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Polyamine patterns in the cerebrospinal fluid of patients with Parkinson's disease and multiple system atrophy

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ABSTRACT

Background: Polyamines (PAs) are important modulators of physiological condition, and are associated with neurodegenerative disease. Thus, we investigated the change of PA concentration in the cerebrospinal fluid (CSF) of patients with Parkinson's disease (PD) and multiple system atrophy (MSA).
Methods: CSF samples from patients with PD and MSA were examined by gas chromatography–mass spectrometry in selected ion monitoring mode using *N*-ethoxycarbonyl/*N*-pentafluoropropyonyl derivatives.
Results: PA concentrations were significantly different in patients with PD and MSA compared with those in the normal group. In the PD group, as compared with the MSA group, concentrations of putrescine, N^1-acetylspermidine, and putrescine spermidine^{-1} were significantly increased, whereas the concentration of spermidine was significantly reduced.
Conclusions: These results could be helpful for understanding the complexity of biochemical events in patients with PD and MSA, and may serve as metabolic markers for diagnosis of PD and MSA.

1. Introduction

Parkinson's disease (PD) is a relatively common neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta [1,2]. Parkinsonism is defined by the presence of two of three symptoms, including rigidity, tremor, and hypokinesia. The most common atypical form of parkinsonism, multiple system atrophy (MSA), is a relentlessly progressive neurodegenerative disorder characterized by neuronal cell loss and gliosis in specific areas of the brain, causing various combinations of systemic degeneration [3,4]. Although it is well known that these two disease entities have different underlying mechanisms, including studies of potential causal factors, differential diagnosis of parkinsonian disorders on the basis of clinical signs and symptoms is still difficult due to overlapping symptomatology.

Physiological polyamines (putrescine, cadaverine, spermidine, and spermine), the naturally occurring di, tri-, and tetra amines, are closely related to neuronal cell biochemical activity in the brain, including interaction with neurotransmitter receptors such as the *N*-methyl-D-aspartate receptor, regulation of substances in degenerating cells, ion channels such as K^+ and protection of neuronal cells from oxidative damage [5–10]. Additionally, administration of spermine reduces infarction in cerebral ischemic rat models [11], although the systemic role of altered PAs has not been well studied.

Because altered PA concentrations are important biochemical and physiological indicators of pathological condition in the brain, and accumulation of PAs has a neuroprotective role, we investigated how PAs change in the CSF of patients with PD and MSA. In our previous report, we found that the long-chain omega-3 fatty acid, eicosapentaenoic acid, associated with an inflammation-lowering protective function, was significantly increased in the CSF of patients with PD and MSA compared with controls [12]. Our result strongly suggests that other metabolomes in the CSF are associated with physiological condition in PD and MSA. Therefore, we extended our study to the metabolic patterns of PAs.

In spite of numerous studies investigating PD and MSA, the metabolic patterns of PAs and their role in these diseases are still unknown. Therefore, in this study, simultaneous metabolic profiling analysis of aliphatic and acetylated PAs was performed to examine altered metabolic patterns of PAs in the CSF of patients with these two diseases, and to gain insight into their biochemical associations.

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2. Materials and methods

2.1. Chemicals and reagents

Polyamine standards including 1,6-diaminohexane as internal standard (IS), ethyl chloroformate (ECF), and pentafluoropropionyl anhydride (PFPA) were from Sigma-Aldrich (St. Louis, MO). Diethyl ether, ethyl acetate, and dichloromethane of pesticide grade were obtained from Kanto (Tokyo, Japan). Sodium chloride was from Junsei (Tokyo, Japan), and was washed successively with methanol, acetone, dichloromethane and diethyl ether, followed by drying under a vacuum (100 °C, 1 h). Sodium hydroxide was from Duksan (Seoul, South Korea). All other chemicals were of analytical grade.

2.2. Patients

Six females and three males with PD, aged 65–79 y, were selected from the Department of Neurology in Ajou University Hospital (Suwon, South Korea) and were diagnosed with PD according to United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnosis Criteria. Five females and four males with MSA, aged 47–66 y, were selected, and a diagnosis of MSA was made according to UK Parkinson's Disease Society Brain Bank Clinical Diagnosis Criteria and consensus criteria for clinical diagnosis of probable MSA, as in our previous study [12]. For our control group, the number of healthy subjects was expanded to 24 (16 females and eight males, aged 46–78 y) compared with 13 healthy subjects who participated as controls for our previous report [12]. Informed consent was obtained from all study subjects. PAs in the CSF were determined as their *N*-ethoxycarbonyl/*N*-pentafluoropropionyl (EOC/PFP) derivatives [13].

2.3. Sample preparation for PAs in the CSF

PAs in the CSF were determined as their *N*-EOC/ *N*-PFP derivatives as previously described [13]. Briefly, 0.5 ml of acetonitrile was added to 50 µl of CSF containing 200 ng of IS to remove proteins. After centrifugation, 0.5 ml distilled water was added to the supernatant and adjusted to pH 12 with 5.0 mol/l sodium hydroxide. A two-phase EOC reaction in aqueous phase was immediately conducted in a single step by vortexing for 10 min in 1 ml of dichloromethane with 20 µl of ECF. The mixture was saturated with sodium chloride and extracted with 3 ml of diethyl ether and 2 ml of ethyl acetate in sequence. The combined extracts were evaporated down to 20 µl under a gentle stream of nitrogen at 40 °C with subsequent PFP derivatization by reacting with 20 µl of PFPA at 60 °C for 30 min for analysis by gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring mode.

2.4. Gas chromatographpy–mass spectrometry

GC–MS analysis was performed using an Agilent 6890 gas chromatograph, interfaced with an Agilent 5973 mass-selective detector (70 eV, electron impact mode) and installed with an Ultra-2 cross-linked capillary column (5% phenyl– 95% methylpolysiloxane bonded phase; 25 m × 0.20 mm i.d., 0.11 um film thickness; Agilent Technologies, Atlanta, GA), as in previous reports [12,13].

2.5. Star symbol plotting

PA concentrations in each CSF sample were normalized to corresponding means in the normal group, and each normalized value was plotted as a line radiating from a common central point. The termini of the lines were joined together to produce the star symbol patterns using the program Microsoft Excel (Microsoft, Redmond, WA), as described elsewhere [14,15].

3. Results and discussion

When the present method was applied to the CSF samples of normal subjects, patients with PD and MSA, three aliphatic PAs (putrescine, cadaverine, and spermidine) and four acetylated PAs (N^1-acetylputrescine, N^1-acetylcadaverine, N^1-acetylspermidine, and N^8-acetylspermidine) were positively detected. Large variations in their concentrations were observed within each group (Table 1).

N^1-Acetylcadaverine and cadaverine in eight normal males showed slightly lower (< 8%) concentrations as compared to their 16 female counterparts, whereas the PA concentrations were slightly increased (4–15%) in female group. When we compared the age group of 46–58 y ($n = 10$) and 60–78 y ($n = 14$), we found that N^1-acetylputrescien and N^1-acetylspermidine were lower (<10%) and putrescine alone was slightly (15%) increased in 60–78 y age group, whereas the PA concentrations were similar in both the groups. The PA concentrations of normal group were not significantly different according to the gender and age, which may be attributed to the fact that we have excluded participants with special diets and having any form of diseases in order to limit any variation. On the other hand we, however, cannot exclude the possibility that this data resulted from inadequate sample size, as relatively few participants met criteria for clinical diagnosis.

In the normal group, cadaverine, which is synthesized by lysine decarboxylase from lysine is the most abundant, followed by N^1-acetylcadaverine and N^8-acetylspermidine. In the PD and MSA groups, cadaverine was also the most abundant, followed by N^8-acetylspermidine and N^1-acetylcadaverine. Normal mean concentrations of putrescine and spermidine in CSF were similar to those described in the literature [16], while other PAs have not been reported.

Table 1
Levels of polyamines in cerebrospinal fluid from patients with Parkinson's disease and multiple system atrophy.

No	Polyamine	Concentration (nmol ml^{-1})			*P*-value[e]	Normalized mean value[f]	
		Mean ± SD (*P* value[a])					
		Normal ($n = 24$)[b]	PD ($n = 9$)[c]	MSA ($n = 9$)[d]		PD	MSA
1	N^1-Acetylputrescine	0.12 ± 0.03	0.12 ± 0.10 (< 0.5)	0.08 ± 0.05 (< 0.001)	< 0.1	0.99	0.63
2	N^1-Acetylcadaverine	0.46 ± 0.09	1.40 ± 0.70 (< 0.001)	1.55 ± 1.26 (< 0.001)	< 0.4	3.04	3.37
3	Putrescine	0.13 ± 0.02	0.20 ± 0.02 (<0.001)	0.13 ± 0.04 (< 0.3)	< 0.001	1.61	1.05
4	Cadaverine	2.12 ± 1.48	3.34 ± 1.03 (< 0.02)	3.62 ± 1.49 (< 0.007)	< 0.3	1.57	1.71
5	N^1-Acetylspermidine	0.16 ± 0.03	0.16 ± <0.01 (< 0.4)	0.11 ± 0.03 (< 0.001)	< 0.001	0.99	0.67
6	N^8-Acetylspermidine	0.25 ± 0.03	0.38 ± 0.14 (<0.001)	0.52 ± 0.50 (< 0.005)	< 0.2	1.51	2.07
7	Spermidine	0.12 ± 0.01	0.07 ± 0.01 (<0.001)	0.11 ± 0.04 (< 0.2)	< 0.003	0.61	0.95
	Total polyamines	3.37 ± 1.49	5.68 ± 1.60 (< 0.001)	6.13 ± 1.71 (< 0.001)	< 0.3		
	Putrescine Spermidine^{-1}	1.06 ± 0.19	2.80 ± 0.24 (<0.001)	1.19 ± 0.20 (< 0.05)	< 0.001		

[a] Student's *t*-test at 95% confidence level on the mean values of normal, PD and MSA groups. [b] Normal group (16 females and eight males, aged 46–78 y). [c] PD group (six females and three males, aged 65–79 y). [d] MSA group (five females and four males, aged 47–66 y). [e] Student's *t*-test at 95% confidence level on the mean values for the PD and MSA groups. [f] Values normalized to corresponding normal mean values.

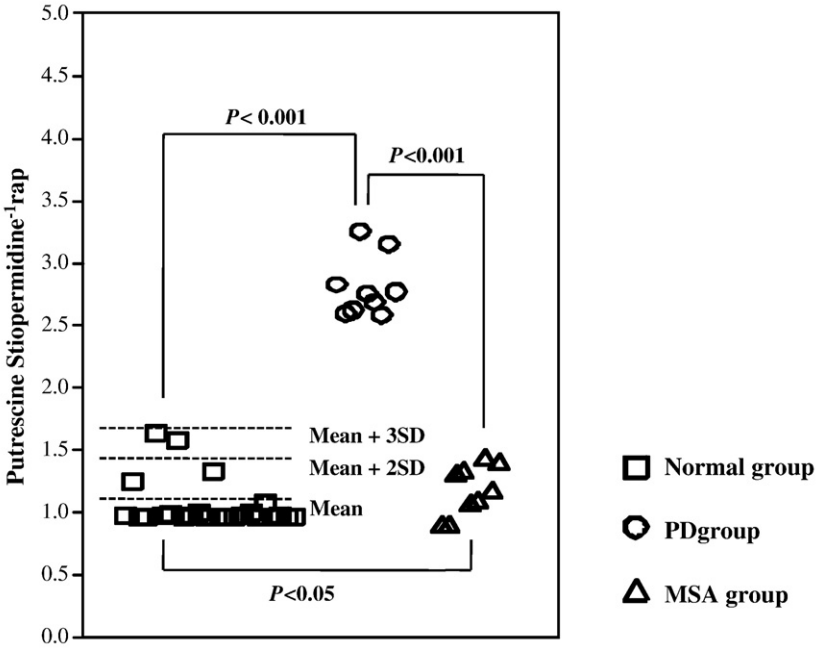

Fig. 1. Scatter plots of the putrescine spermidine^{-1} ratio in CSF samples from normal subjects, PD and MSA patients.

According to Student's *t*-tests of the means of the normal and two disease groups, concentrations of N^1-acetylcadaverine ($P<0.001$), putrescine ($P<0.001$), cadaverine ($P<0.02$), N^8-acetylspermidine ($P<0.001$), total PAs ($P<0.001$) and putrescine spermidine^{-1} ($P<0.001$) were significantly increased, whereas the concentration of spermidine ($P<0.001$) was significantly reduced in the PD group (Table 1). In the MSA group, concentrations of N^1-acetylcadaverine ($P<0.001$), cadaverine ($P<0.007$), N^8-acetylspermidine ($P<0.005$), total PAs ($P<0.001$), and putrescine spermidine^{-1} ($P<0.05$) were significantly increased, while concentrations of N^1-acetylputrescine ($P<0.001$) and N^1-acetylspermidine ($P<0.001$) were significantly reduced in the MSA group, as compared to the normal group (Table 1). It is interesting to note that the concentration of N^1-acetylcadaverine is highly increased in the CSF of PD and MSA patients, and mainly metabolized by monoamine oxidase in mitochondria [17]. Dysfunctional mitochondria, a well-known cause of PD, can deregulate the concentration of N^1-acetylcadaverine.

Putrescine concentrations in particular, N^1-acetylspermidine and putrescine spermidine^{-1}, were significantly increased, while spermidine concentration was significantly reduced in the PD group, as compared with those of the MSA group (Table 1). The lower concentration of spermidine and the higher concentration of the ratio of putrescine to spermidine in the PD group may explain the accumulation of putrescine and the reduced metabolic rate for spermidine biosynthesis from putrescine as a precursor of a deregulated metabolic pattern. Although the biochemical mechanisms responsible for these peculiar CSF PA profiles are unclear, disturbances in PA metabolism may be useful as potential biochemical diagnostic markers in patients with PD and MSA [6,18].

The scatter plot was drawn based on putrescine spermidine^{-1} values for the nine PD patients (open circles) and lower values for the nine MSA patients (Δ) compared with that (mean + 3 SD) of 24 normal controls (□), as demonstrated in Fig. 1. This result may explain differing PA metabolisms between PD and MSA patients. In addition, the relatively high concentrations of putrescine spermidine^{-1} ratio in CSF from PD group compared with MSA group are considered to be responsible for selective neuronal degeneration in substantia nigra (SN), since MSA shows much more progressive and widespread neurodegeneration in basal ganglia, cerebellum, spinal cord and brainstem. It is further suggested that the possibility of deregulation of PA metabolisms which regulate polyamine homeostasis should be investigated with an emphasis on the unknown etiology or pathogenesis between PD and MSA.

When the PA concentrations in each sample from the PD and MSA groups were normalized to the corresponding normal mean value,

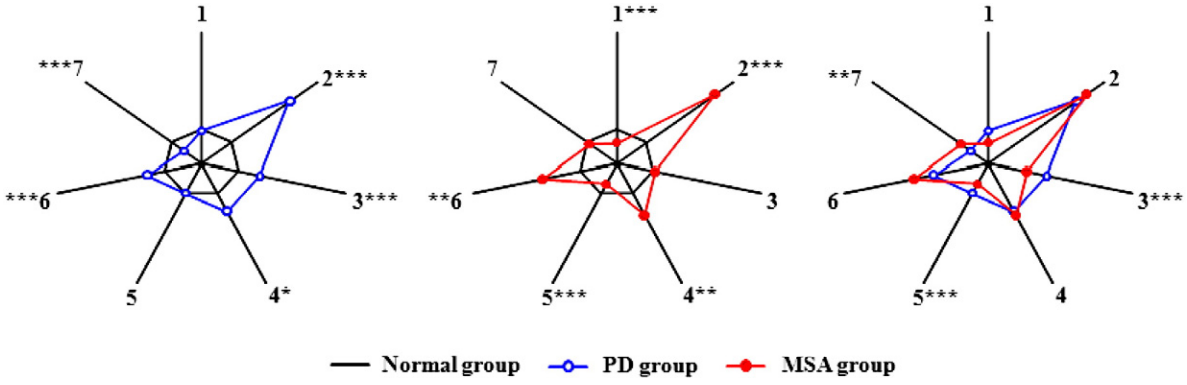

Fig. 2. Star symbol plots of normal, PD, and MSA groups. Numbers on the rays correspond to those in Table 1. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, Student's *t*-test at the 95% confidence concentration.

they were informative in expressing the increased PA concentrations in multiples (range in PD: 0.61–3.04, range in MSA: 0.63–3.37) of the normal mean values (Table 1). These concentrations were found to be useful in discriminating patient groups from the normal group. When these normalized values were used as variables to draw star graphs composed of seven rays to visually illustrate PA differences among normal, PD, and MSA, groups were clearly distinct (Fig. 2) compared with numerical data (Table 1), and readily distinguishable from the heptagon shape for the normal group mean, which was previously shown to be an adequate reference pattern [14,15]. Although the normal group mean proved adequate as a control pattern for the patient group, a pressing need exists for large-scale studies of PA change in the CSF of patients with PD and MSA in order to clarify the significance of PA metabolism in these diseases. Thus, the present metabolic profiling analysis of PAs in CSF, combined with a graphic analysis, may be useful as a clinical tool for biochemical monitoring of patients with PD and MSA. Furthermore, our study may be helpful for elucidating PA alteration of CSF on other neurodegenerative diseases such as motor neuron disease, Alzheimer disease or the ageing brain since it has been very poorly addressed on such instances.

In conclusion, we demonstrated that PA concentrations in the CSF of patients with PD and MSA exhibit several significant differences, along with differing trends. These results could be important in the understanding of systematic function and development of more efficient bioassays for diagnosis of MSA and PD. These results also suggest that altered PAs are related to development of MSA or PD. Our CSF PA profiling combined with star graphic analysis may be a generally useful clinical tool, not only for monitoring patients with MSA and PD, but also for other neurological patients with distinct biochemical profiles.

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