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Associations between plasma ceramides and cognitive and neuropsychiatric manifestations in Parkinson's disease dementia

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ABSTRACT

Background: The abnormal metabolism of ceramides may account for the pathogenesis of Parkinson's disease dementia (PDD). However, the effect of ceramides on cognitive domain impairments and neuropsychiatric symptoms of PDD remains unknown.
Methods: A total of 38 PDD, 40 PD with no cognitive impairment (PD-NC) and 40 normal controls were included. A series of cognitive tests and the Neuropsychiatric Inventory (NPI) were used to assess cognitive domains and neuropsychiatric symptoms. A non-fasting blood sample was obtained from each subject. Plasma ceramide levels were tested by HPLC–MS/MS analysis.
Results: C14:0 and C24:1 levels were significantly higher in PDD than in PD-NC and normal controls. Verbal memory was negatively correlated with C14:0 and C24:1. After controlling for confounding factors, C22:0, C20:0 and C18:0 were significantly associated with hallucinations, anxiety and sleep behavior disturbances, respectively.
Conclusion: In PDD, the increase in ceramide levels was correlated with decreased memory function and associated with higher odds of multiple neuropsychiatric symptoms.

1. Introduction

It has been reported that approximately 80% of Parkinson's disease (PD) patients developed dementia (PDD) during an 8–20-year follow-up period [1,2]. In addition to cognitive impairment, PDD patients commonly exhibit neuropsychiatric symptoms; indeed, up to 89% of PDD patients presented at least one neuropsychiatric symptom [3]. Cognitive impairment and neuropsychiatric symptoms significantly exacerbate disabilities in these patients and increase the burden on caregivers. The exact mechanisms of PDD remain unclear. Previous studies have suggested that ceramides are likely one of the factors influencing the pathogenesis of PDD.

Ceramides, the core constituents of sphingolipids, play crucial roles in various cell physiological functions, including apoptosis, growth arrest, senescence, migration and adhesion [4]. In PDD, the abnormal accumulation of brain α-synuclein is the major neuropathological change, and nearly half of PDD patients also show extensive amyloid-β plaques (Aβ), which synergistically act with α-synuclein to develop PDD [5]. Previous studies suggested that ceramides were associated with both α-synuclein and Aβ accumulations. Pathological studies showed that ceramides were selectively altered in brain regions where α-synuclein accumulated [6,7]. The disruption of ceramide homeostasis in the endoplasmic reticulum dramatically enhances the toxicity of α-synuclein [8]. For Aβ, the exposure of hippocampal neurons to Aβ increased ceramide species, and treatment to prevent this ceramide accumulation protected neurons against Aβ-induced death [9].

Although the aforementioned evidence suggests the likely effects of ceramides on the pathogenesis of PDD, studies on the associations between ceramides and the clinical manifestations of PDD remain insufficient. Only one previous study showed that PDD patients had higher plasma levels of ceramides compared with PD patients with no cognitive impairment (PD-NC) and normal controls [10]. However, the relationship between ceramides and different cognitive domain impairments of PDD remains unknown and should be further investigated. Furthermore, multiple neuropsychiatric disorders were suggested to be associated with the abnormal metabolism of ceramides [11]. Ceramides were significantly increased in brain white matter of patients with neuropsychiatric disorders [12]. The alternations of ceramide metabolism have been observed in people suffering from anxiety and depression [13,14]. Thus, we speculated that plasma ceramide levels are associated with the neuropsychiatric symptoms of PDD.

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In the present study, we first assessed the plasma ceramide levels in PDD patients compared with PD-NC patients and normal controls. Furthermore, we systematically investigated the associations between ceramide species and the cognitive and neuropsychiatric manifestations of PDD.

2. Methods

2.1. Subjects

All of the subjects were recruited from the Department of Neurology at Xuan Wu Hospital. The diagnosis of PD was based on the UK PD Brain Bank Criteria [15]. PDD was diagnosed according to the Movement Disorder Society criteria [16]. All controls had normal cognitive abilities, with no previous history of neurological disorders. Written informed consent was previously obtained from all participants or their relatives. This study was approved by the Institutional Review Board of Xuan Wu Hospital.

2.2. Assessments

The Unified Parkinson's Disease Rating Scale (UPDRS) part III was used to evaluate motor disability. The Mini-Mental State Examination (MMSE) [17] and Clinical Dementia Rating scale (CDR) [18] were used to assess global cognitive ability and dementia severity. All PDD patients in the present study underwent more detailed cognitive and neuropsychiatric assessments. All assessments were conducted during the "on" motor state. The Digit span task and Trail Making Test were used to evaluate attention and working memory. The Boston Naming test, World Health Organization-University of California-Los Angeles Auditory Verbal Learning Test (WHO-UCLA AVLT) [19] and Clock Drawing Test were used to examine language, memory and visuospatial and executive function, respectively.

The Neuropsychiatric Inventory (NPI) was used to determine neuropsychiatric symptoms [20]. The NPI was scored based on information obtained from the caregivers. The NPI includes the following symptoms: delusions, hallucinations, agitation/aggression, apathy, anxiety, depression, euphoria, disinhibition, irritability, aberrant motor behavior, sleep behavior disturbances, and appetite abnormalities. If a patient did not have any of these symptoms in the previous month, then the NPI was scored as 0. If the answer was "yes", then the frequency and severity were assessed. The score of each symptom was calculated as the product of the frequency and severity (maximum score = 12).

The lysosomal enzyme glucocerebrosidase (GBA) hydrolyzes glucosylceramide into glucose and ceramide. Thus, mutations in the GBA gene, which has been regarded as a genetic risk factor for PD, may influence plasma ceramide levels [21]. L444P and N370S heterozygotes are the most common and clinically important variants in GBA gene. For all our subjects, polymerase-chain-reaction (PCR) assay was used to identify the L444P and N370S mutations. The PCR primers, annealing temperatures and restriction enzymes were according to the previous study [22].

2.3. HPLC–MS/MS analysis

A non-fasting blood sample was obtained from each subject and maintained at −80 °C until further analysis. The ceramide assays were conducted at the Institute of Materia Medica, Peking Union Medical College (Beijing, China). A three-phase solvent system was used to prepare the samples. HPLC–MS/MS was performed on an Agilent 6410B Triple Quad mass spectrometer (QQQ; Agilent Technologies, Santa Clara, CA, USA), comprising a triple quadrupole MS analyzer with an electrospray ionization (ESI) interface and an Agilent 1200 RRLC system. A Spectra C8SR Column (150 × 3.0 mm; 3 μm particle size; Peeke Scientific, Redwood City, CA) was used to perform chromatographic separation. The following parameters were used for electrospray ionization tandem mass spectrometry (ESI-MS/MS): polarity = positive, gas temperature = 350 °C, gas flow = 6 l/min, nebulizer = 15 psi, capillary = 4000 V. All standards were from Avanti Polar Lipids (Alabaster, AL, USA). The calibration mixtures for the standard curves were prepared at concentrations of 250 pmol/ml (Std A) and 2000 pmol/ml (Std B) in methanol. The calibration curve standards were prepared using blank matrix, which was about 1 mg BSA dissolved in 0.1 ml tissue homogenate buffer solution. All calibration curves comprised eight calibration points covering concentrations of 2.5, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 400.0 pmol/mg protein, and were constructed by spiking Std A or Std B. Quality control (QC) samples with low, middle and high concentration at 10.0, 50.0, 200.0 pmol/mg protein were also prepared in the way same as the calibration curves. All calibration curves were constructed by the peak area ratios of the analyte to its corresponding internal standard against the eight calibration point concentration. The detailed procedures for standard curves and validation results for precision, accuracy and absolute recovery had been previously published [23].

2.4. Statistical analysis

The Kolmogorov-Smirnov test was used to determine normality. For demographic data, χ^2 tests were conducted to compare group differences for dichotomous variables. Continuous data were analyzed using one-way ANOVA or Welch's test to identify overall differences between the groups, with Tukey's or Games-Howell post hoc tests to identify individual group differences. For ceramide levels, comparisons among groups were analyzed using the Kruskal-Wallis test followed by Mann-Whitney U tests. The correlations between plasma ceramide levels and scores of cognitive and neuropsychiatric assessments were analyzed with Spearman tests. Furthermore, for the cognitive scores which were correlated with ceramides, linear regression models were constructed. The linear regressions were adjusted for age, CDR, diabetes status, total NPI score, the use of cholinesterase inhibitors and levodopa equivalent dose. Logistic regression analyses were performed to examine the associations between ceramide species and individual neuropsychiatric symptoms. The levels of each ceramide species were separately added to the regression models as independent variables. The logistic regression analyses were adjusted for age, CDR, diabetes status, and medications. A p value <0.05 was considered statistically significant.

3. Results

3.1. Demographic data and plasma ceramide levels

A total of 38 PDD patients, 40 PD-NC patients and 40 normal controls were included in the present study. The PDD patients were with mild (CDR = 1, $n = 26$) or moderate (CDR = 2, $n = 12$) dementia. The characteristics of the subjects are presented in the Table 1. Cholinesterase inhibitors were used in 12 (30%) PDD patients. No patient was receiving antipsychotics or antidepressants. None of the L444P and N370S mutations were present in the DNA obtained from our subjects. For group comparisons of ceramide levels, the levels of C22:0 and C24:1 were significantly higher in PD-NC patients than in normal controls, while PDD patients showed even higher levels of C24:1 compared with PD-NC patients. C14:0 levels, which were comparable between PD-NC patients and controls, were significantly higher in PDD patients than in the other two groups (Fig. 1).

3.2. Correlations between ceramides and the scores of cognitive and neuropsychiatric assessments in PDD patients

The cognitive and neuropsychiatric test scores obtained from PDD patients are shown in Table 2. For global cognitive function, no correlations were observed between MMSE and plasma ceramides in PD-NC and PDD. However, significant negative correlations were observed