Parkinsonism and Related Disorders xxx (2015) 1–6

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Reduced plasma serotonin and 5-hydroxyindoleacetic acid levels in Parkinson's disease are associated with nonmotor symptoms

Qing Tong [a, 1], Li Zhang [a, 1], Yongsheng Yuan [a, 1], Siming Jiang [a], Rui Zhang [b], Qinrong Xu [a], Jian Ding [a], Daqian Li [c], Xiaobin Zhou [d], Kezhong Zhang [a, *]

[a] Department of Neurology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[b] Department of Neurosurgery, Nanjing Children's Hospital of Nanjing Medical University, Nanjing 210029, China
[c] Department of Laboratory Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[d] Department of Microbiology and Immunology, Nanjing Medical University, Nanjing 210029, China

ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form
3 May 2015
Accepted 25 May 2015

Keywords:
Serotonin
5-Hydroxyindoleacetic acid
Depression
Pain
Parkinson's disease

ABSTRACT

Background: Accumulating evidence suggests that serotonergic system may be implicated in the pathophysiology of Parkinson's disease (PD), and particularly in nonmotor symptoms such as depression, fatigue, sleep disorders, sensory and autonomic dysfunction. This study aimed to evaluate plasma levels of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in PD patients, and investigate their associations with nonmotor symptoms.

Methods: Eighty-two PD patients and sixty-four controls underwent a series of clinical assessments, including Hamilton Depression Scale, Fatigue Severity Scale, Pittsburgh Sleep Quality Index, Visual Analog Scale for Pain, and Scale for Outcomes in PD for Autonomic Symptoms. Plasma 5-HT and 5-HIAA levels were measured by HPLC-ECD.

Results: PD patients exhibited worse performance on nonmotor symptom scales (all P-values <0.001) and presented lower plasma levels of 5-HT ($P < 0.001$) and 5-HIAA ($P < 0.001$) than control individuals. Within the PD group, decreased concentrations of plasma 5-HT and 5-HIAA were correlated with more severe depression ($r = -0.447$, $P < 0.001$; $r = -0.407$, $P < 0.001$, respectively) and pain ($r = -0.485$, $P < 0.001$; $r = -0.416$, $P < 0.001$, respectively). After performing multiple linear regression, plasma 5-HT ($P = 0.01$) and 5-HIAA ($P = 0.006$) remained significantly associated with depression.

Conclusions: Our results suggest that serotonergic dysfunction might exist in PD, and specifically correlated with depression and pain in PD. Plasma levels of 5-HT and 5-HIAA may be considered as peripheral markers for depression in PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, of which the main pathological hallmark is the loss of dopamine neurons in the substantia nigra. Clinically, PD is characterized by motor features including bradykinesia, rigidity, tremor, and postural instability. However, the classic view of PD as a pure dopaminergic and movement disorder is changing. Experimental and clinical findings have shown that serotonergic system might be involved in the pathophysiology of PD [1,2]. Additionally, a wide spectrum of nonmotor symptoms (NMS), such as mood disturbances, fatigue, sleep disorders, sensory and autonomic dysfunction, also frequently occur in PD patients, which are considered to be disabling and contribute to poor quality of life [3,4].

The postmortem study has shown that the neuropathological process of PD extends beyond the striatum and dopaminergic system [5]. According to Braak's hypothesis, during the early stages the Lewy bodies occur in the raphe nuclei, which is responsible for the production of serotonin (5-HT), indicating a potential role of serotonergic system in the pathophysiology of PD [5]. Additional support for this concept comes from another postmortem study that observed the depletion of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in PD patients [6]. Moreover, previous in vivo neuroimaging studies have

* Corresponding author. Department of Neurology, The First Affiliated Hospital of Nanjing Medical University, No. 300 Guangzhou Road, Nanjing 210029, China.
E-mail address: kezhong_zhang1969@126.com (K. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.parkreldis.2015.05.016
1353-8020/

reported significant reductions of serotonin transporter binding in the brainstem of PD patients [7,8]. Recently, Olivola et al. found the cerebrospinal fluid (CSF) levels of 5-HT and 5-HIAA in PD patients were markedly lower than those in controls [9]. Interestingly, there are data to suggest that serotonergic dysfunction may be implicated not only in PD, but also more specifically in the occurrence of NMS [10]. Emerging evidence from biochemical, animal models and human studies has demonstrated the involvement of serotonergic system in the pathophysiology of NMS in PD, including depression, fatigue, sleep disorders, pain, as well as autonomic dysfunction [11,12]. Furthermore, several open label studies have shown a beneficial effect of serotonergic agents on the treatment of motor and nonmotor symptoms in PD patients [2,13]. However, none of these studies attempted to investigate whether serotonin markers in plasma are altered in patients with PD and associated with such symptoms. Therefore, in this study, we sought to assess levels of 5-HT and 5-HIAA in plasma of PD patients, and evaluate their associations with NMS, which in turn may provide evidence for the use of agents acting on serotonergic neurotransmission.

2. Methods

2.1. Patients

Eighty-two patients with idiopathic PD, including newly diagnosed and advanced patients, were recruited consecutively from the Department of Neurology of the First Affiliated Hospital of Nanjing Medical University, China, between August 2012 and June 2013. All patients were diagnosed by two experienced neurologists according to the UK Parkinson's Disease Society Brain Bank criteria, with the disease duration ranged from 0.5 to 18 years. Exclusion criteria were: neurological diseases other than PD, severe hearing or visual loss, or other conditions that might interfere with the reliable completion of clinical assessments. As control group, sixty-four age- and gender-matched individuals without a family history of PD and neurological diseases were also recruited from our institution. Both patients and controls were included only if they were volunteered to participate in scientific studies, and if no signs of cognitive impairments were detectable by the Mini-Mental State Examination (MMSE score <24). PD patients included in our study had a stable response to their anti-parkinsonian medications. Moreover, none of the participants was taking medications acting on serotonergic system. This study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University and all subjects provided written informed consent.

2.2. Clinical assessment

All patients were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr staging scale (H&Y) during their off state. In addition, Levodopa-equivalent daily dose (LEDD), which served to establish the anti-parkinsonian medications, was calculated according to conversion factors as described previously [14]. All study participants were subjected to a thorough clinical assessment, which included validated measures for the evaluation of NMS in PD patients. The severity of depressive symptoms was assessed with the Hamilton Depression Scale (HAMD), in which the cut-off value for screening purposes is 9/10 [15]. Fatigue was evaluated by using the Fatigue Severity Scale (FSS) and patients with a score ≥ 4 were defined as suffering from significant fatigue. Sleep disorder was assessed using the Pittsburgh Sleep Quality Index (PSQI), with a score > 5 indicating the presence of sleep disturbance. The pain severity was recorded with the Visual Analog Scale (VAS) for Pain. Finally, the autonomic dysfunction was evaluated using the Scale for Outcomes in PD for Autonomic Symptoms (SCOPA-AUT).

2.3. Plasma sampling and biological assays

Venous blood samples were drawn in vacuum tubes containing EDTA in the morning on the same day of clinical assessment, after an overnight fast. Considering the full vanishing of the effect of prolonged released dopamine agonists, venipuncture was conducted in PD patients after three-day withdrawal of anti-parkinsonian medications. From another aspect, five days or more was considered to be unethical by the ethics committee. Blood samples were immediately centrifuged at 3,000 g for 15 min at 4 °C, and plasma was aliquoted and stored at −80 °C until analysis. At the time of analysis, all plasma samples were deproteinized with trichloroacetic acid (1: 0.24 V/V) after thawing, and immediately centrifuged at 20,000 g for 30 min at 4 °C. The protein-free supernatants were then measured by HPLC-ECD. Briefly, the HPLC-ECD system consisted of a Thermo UniMate 3000 pump, an UniMate 3000 autosampler, and an ESA Coulochem III electrochemical detector (working electrode +350mv). Thermo BDS HYPERSIL column (4.6 × 250 mm, 5 μm) was used at 38 °C. The mobile phase (90 mmol/L NaH_2PO_4, 50 mmol/L citric acid, 1.7 mmol/L OSA, 50umol/L EDTA, 10% acetonitrile) was delivered to the analytical column at a rate of 1.0 ml/min. Chromeleon 6.8 software was used for data collection and processing.

2.4. Statistical analysis

Statistical analyses were performed with SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). All data were tested for normality of distribution by using Shapiro–Wilk test ($P > 0.05$). Comparisons between groups were made using Chi-squared test for categorical

Table 1
Demographic and clinical features of PD patients and control subjects.

	PD (n = 82)	Control (n = 64)	P
Age (years), mean ± SD	60.88 ± 10.77	63.92 ± 10.60	0.09[a]
Male: females	29:53	28:36	0.303[b]
Disease duration (years), mean ± SD	3.35 ± 3.62	–	–
UPDRS score, mean ± SD	27.67 ± 13.60	–	–
UPDRS I score, mean ± SD	2.66 ± 1.83	–	–
UPDRS II score, mean ± SD	11.28 ± 6.30	–	–
UPDRS III score, mean ± SD	13.85 ± 7.75	–	–
Hoehn and Yahr stage, mean ± SD	1.82 ± 0.76	–	–
LEDD (mg/day), mean ± SD	201.14 ± 331.72	–	–
MMSE score, mean ± SD	27.35 ± 3.14	28.11 ± 2.85	0.357[c]
HAMD score, mean ± SD	11.13 ± 7.59	0.86 ± 0.94	<0.001[c]
FSS score, mean ± SD	4.19 ± 1.60	1.93 ± 0.68	<0.001[c]
PSQI score, mean ± SD	5.78 ± 3.74	0.31 ± 0.59	<0.001[c]
VAS score, mean ± SD	3.87 ± 2.25	0.39 ± 0.88	<0.001[c]
SCOPA-AUT score, mean ± SD	11.68 ± 9.23	0.64 ± 0.97	<0.001[c]
5-HT (μg/L), mean ± SD	26.53 ± 15.07	42.58 ± 16.99	<0.001[c]
5-HIAA (μg/L), mean ± SD	96.48 ± 51.70	186.75 ± 99.12	<0.001[c]

PD, Parkinson's disease; SD, Standard deviation; UPDRS, Unified Parkinson's disease rating scale; LEDD, levodopa-equivalent daily dose; MMSE, Mini-Mental State Examination; HAMD, Hamilton Depression Scale; FSS, Fatigue Severity Scale; PSQI, Pittsburgh Sleep Quality Index; VAS, Visual Analog Scale for Pain; SCOPA-AUT, Scale for Outcomes in PD for Autonomic Symptoms; 5-HT, serotonin; 5-HIAA, 5-hydroxyindolacetic acid.
[a] Student's t-test.
[b] Chi-square test.
[c] Mann–Whitney test.

Please cite this article in press as: Q. Tong, et al., Reduced plasma serotonin and 5-hydroxyindoleacetic acid levels in Parkinson's disease are associated with nonmotor symptoms, Parkinsonism and Related Disorders (2015), http://dx.doi.org/10.1016/j.parkreldis.2015.05.016