SERUM HOMOCYSTEINE IN LEVODOPA TREATED PARKINSON’S DISEASE PATIENTS

MARIA DRONCA1*, SERGIU-PETRICĂ PAŞCA1, RĂZVAN RUSU1, LĂCRĂMIOARA PERJU-DUMBRAVĂ2, NICOLETA TOHÂNEAN2

1Biochemistry Department, “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania
2Neuroscience Department, “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania

(Received June 19, 2008)

Hyperhomocysteinemia is strongly associated with an increased risk of dementia and cognitive impairment, both of which are common in the course of Parkinson’s disease (PD). Although B-vitamins’ status and genetic factors strongly influence the degree of this elevation, an increased level of total serum homocysteine (tHcy) may also be due to metabolism of levodopa, an intermediate in the biosynthesis of the neurotransmitter dopamine. The aim of this study was to determine whether hyperhomocysteinemia is associated with the advanced stage of PD and with the administered dose of levodopa, in levodopa treated PD patients. Twenty five PD patients, receiving levodopa as substitution therapy, and 28 healthy control subjects, age and sex matched, were included in this study. tHcy level was significantly higher in the patient group in comparison with the control group (14.01±0.95 µM vs. 11.44±0.80 µM; P=0.04) and there was a positive correlation between tHcy level and both the disease stage (r=0.43; P<0.05) and the daily levodopa dose, the latter being statistically significant (r=0.47; P=0.02) when applying a correction for age.

Key words: homocysteine, levodopa, Parkinson’s disease.

INTRODUCTION

Parkinson's disease (PD) is one of the most studied and best understood neurological disorders. PD patients exhibit motor symptoms such as resting tremor, rigidity and bradykinesia (1), as well as non-motor abnormalities (2). The immediate cause of PD is neural degeneration in the substantia nigra area, a dopaminergic movement center (3). As the dopamine-producing cells are lost, walking, arm movement and facial expression are affected.

* Corresponding author (E-mail: m_dronca@yahoo.com)

ROM. J. BIOCHEM., 45, 2, 141–147 (2008)
Levodopa, or L-Dopa (3,4-dihydroxy-L-phenylalanine), is the most effective drug for the symptomatic management of PD. However, its long-term use is limited by the development of motor complications that affect the large majority of PD patients. Levodopa is used to increase dopamine levels, since it is able to cross the blood-brain barrier, whereas dopamine cannot. After crossing the barrier, levodopa is taken up by surviving striatal neurons, converted by intraneuronal aromatic L-amino acid decarboxylase into dopamine, a neurotransmitter that is released from the presynaptic neuronal terminals (4).

Elevated levels of total plasma homocysteine (tHcy), meaning the sum of protein-bound and free Hcy, were reported in patients with PD (5–7). Several cross-sectional studies (8–14) suggested that the increase in Hcy is confined to those PD patients receiving levodopa. Prospective studies (15, 16) and animal studies (17, 18) also supported L-Dopa as an acquired cause for elevated Hcy plasma levels.

Given the inconsistencies in the literature regarding the relationship between levodopa treatment and elevated tHcy levels (7, 9, 11, 16–20), we investigated the serum concentration of tHcy in levodopa-treated PD patients in comparison to age and sex matched healthy controls, in order to determine whether hyperhomocysteinemia is associated with the dose of levodopa and the advanced stage of the disease.

MATERIAL AND METHODS

SUBJECTS

Twenty five PD patients hospitalized in the Clinic of Neurology, Cluj-Napoca, and 28 age and sex matched control subjects, free of any neurological conditions, were included in this study. PD patients were at a mean Hoehn and Yahr’s stage (21) of 2.66±0.12. Global cognitive functions were assessed using Mini-Mental State Examination (22) and the mean value was 27.72±0.48. Informed consent was obtained and the research protocol was in agreement with the Declaration of Helsinki of the World Medical Association.

METHODS

Blood samples were collected from each individual after overnight fasting. All serum specimens were stored at −20°C before Hcy was determined. Total serum Hcy was assessed by reversed-phase high performance liquid chromatography with pre-column derivatization and fluorescence detection, by using Agilent chromatographic system and Chromsystems kit (Chromsystems Instruments & Chemicals GmbH, München, Germany).
STATISTICS

The data were presented as means ± standard errors. Student t-test or the Mann-Whitney rank-sum test was used to compare the tHcy level in patients and their age and sex matched controls. Spearman's rho test was used to evaluate the correlation between different variables. A probability (P) value of less than 0.05 was considered statistically significant. The SPPS software (version 13.0) was used for performing the statistical analysis.

RESULTS

PD and control subjects were homogeneous for age and sex distributions (Table 1).

Table 1
Characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Parkinson’s disease</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>25</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Male subjects</td>
<td>9 (36%)</td>
<td>10 (40%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>63.44±1.74</td>
<td>60.17±1.57</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

The mean duration of PD was 3.92±0.34 years and the mean duration of levodopa treatment (404.0±34.63 mg/day) was 2.84±0.29 years.

The mean serum tHcy level was significantly higher in PD subjects treated with levodopa than in controls (14.01±0.95 µM vs. 11.44±0.80 µM; P<0.05) (Figure 1).

Fig. 1. – Homocysteine level in Parkinson’s disease (PD) and control (C) patients.
There was a positive correlation between the tHcy level and the Hoehn and Yahr stage ($r=0.43; P<0.05$). We observed a positive correlation between tHcy level and levodopa dose (Figure 2), but at a borderline statistical level of significance ($r=0.35, P=0.08$); this correlation became statistically significant after applying a correction for age ($r=0.47; P=0.02$).

![Graph showing the relationship between levodopa dose and total serum homocysteine level in Parkinson’s disease patients.](image)

**DISCUSSION**

Our study clearly shows an association between levodopa therapy and the tHcy level. This finding is in agreement with previous reports (7, 20, 23) and prove that hyperhomocysteinemia is caused by levodopa treatment rather than by PD *per se*. The higher doses of levodopa, administered in the more severe cases of PD, result in increased plasma Hcy levels and explain the positive correlation between tHcy level and the Hoehn and Yahr stage.

The most likely mechanism underlying hyperhomocysteinemia occurrence in levodopa treated PD patients involves O-methylation of levodopa, catalyzed by catechol-

*O*-methyltransferase (the levodopa metabolism). This enzyme uses S-adenosyl-
methionine as methyl donor and produces S-adenosylhomocysteine, which is rapidly hydrolyzed by S-adenosylhomocysteine hydrolase to Hcy (18, 24) (Figure 3). Another pathway involved in levodopa-induced hyperhomocysteinemia is the reduced remethylation of Hcy, due to a deficiency of folic acid (vitamin B9) and vitamin B12 or to a genetic inefficiency of the enzymes methionine synthase and methylene tetrahydrofolate reductase (MTHFR) involved in this transformation. Dysfunction in Hcy clearance by transsulfuration pathway, induced by vitamin B6 deficiency, by lack of allosteric activation of cystathione β-synthase by S-adenosylmethionine, or by the genetic inefficiency of this enzyme (25), can also contribute to the hyperhomocysteinemia observed in these patients. In other words, status of vitamin B12, vitamin B6 and folate (16, 26, 27), as well as activity of MTHFR (8, 12) may modulate levodopa-induced hyperhomocysteinemia in PD patients. Two observations supported this hypothesis: first, levodopa-induced increase in plasma Hcy was lower in the patients from the United States, where folate is added to cereals since 1998 (7), and second, the Hcy elevation was greater in Japanese patients showing a higher frequency of the TT MTHFR genotype (15).

While serum Hcy level is regulated by synthesis and clearance in peripheral tissues, the mechanisms controlling the Hcy level in the central nervous system are
mostly unknown. Hcy can be rapidly taken up by the neurons via specific membrane transporters (28). It could hasten the progression of PD via multiple neuropathological mechanisms, including excessive production of free radicals and cytosolic accumulation of calcium ions, activation of apoptotic pathways and over-stimulation of N-methyl-D-aspartate receptors (29–31). Oxidative stress could play a prominent role in the degeneration of dopaminergic neurons in PD patients (32).

Insurance of normal or higher levels of folic acid and vitamin B_{12} in the PD patients, through intake of food rich in these vitamins or direct supplementation, may prevent the increase of blood Hcy level. Moreover, Hcy should be carefully monitored in these patients during levodopa treatment, and the increased level of Hcy should be adjusted by adding catechol-O-methyltransferase inhibitors or by supplementing with B-vitamins rich diet.

REFERENCES


