Plasma Homocysteine and l-DOPA Metabolism in Patients with Parkinson Disease, Fabio Blandini,1* Roberto Fancellu,1,3 Emilia Martignoni,2,4 Anna Mangiagalli,1 Claudio Pacchetti,2 Alberta Samuele,3 and Giuseppe Nappi2,5 (1 Laboratory of Functional Neurochemistry, 2 Center for Parkinson Disease and Movement Disorders, Neurological Institute “C. Mondino”, 27100 Pavia, Italy; 3 University of Insubria, 21100 Varese, Italy; 4 University of Piemonte Orientale “Amedeo Avogadro”, 28100 Novara, Italy; 5 Institute of Nervous and Mental Diseases, University of Rome “La Sapienza”, 00185 Rome, Italy; * address correspondence to this author at: Laboratory of Functional Neurochemistry, Neurological Institute “C. Mondino”, Via Palestro, 3 27100 Pavia, Italy; fax 39-0382-380286, e-mail fabio.blandini@mondino.it)

Homocysteine is formed by demethylation of methionine and is involved in transmethylation mechanisms. Hyperhomocysteinemia is associated with a wide range of clinical manifestations, mostly affecting the central nervous system (e.g., mental retardation, cerebral atrophy, and epileptic seizures) (1, 2). Hyperhomocysteinemia has also been associated with an increased risk for atherosclerotic and thrombotic vascular diseases (3–5). Although various mechanisms have been proposed, mostly involving endothelial damage related to increased oxidative stress (6), the exact cellular and molecular bases for the adverse effects of homocysteine are still elusive.

Alterations in transmethylation reactions that lead to hyperhomocysteinemia have been suggested in the pathophysiology of neurodegenerative disorders such as Alzheimer disease and Parkinson disease (PD) (7, 8). Furthermore, the potential role of homocysteine in neurodegeneration has recently been pointed out by a study showing that the amino acid induces apoptosis in cultures of hippocampal neurons (9).

The processes of methyl-group transfer are involved in the metabolism of l-3,4-dihydroxyphenylalanine (l-DOPA), the most effective drug used for the therapy of PD (10). The main metabolism of l-DOPA is its O-methylation to form 3-O-methyl-DOPA (3-OMD). The reaction involves the enzyme catechol-O-methyl-transferase, with S-adenosylmethionine as methyl-group donor. Demethylation of S-adenosylmethionine forms S-adenosylhomocysteine, which is hydrolyzed to homocysteine. Homocysteine is then metabolized via a transsulfuration pathway, forming cystathionine, or a remethylation cycle, which leads back to methionine (11). The catabolism of l-DOPA might therefore interfere, at various steps, with homocysteine metabolism. Indeed, there is experimental evidence that l-DOPA administration increases concentrations of plasma homocysteine (12) and cerebral S-adenosylhomocysteine (12, 13). In PD patients treated with l-DOPA, plasma homocysteine is higher than in controls and untreated PD patients (14–17). This further supports the idea that the drug, rather than the disease per se, promotes hyperhomocysteinemia (17).

The aim of our study was to investigate the relationship between l-DOPA therapy and homocysteine in PD patients. For this purpose, we determined the plasma concentrations of homocysteine in PD patients under regular treatment with the drug and in healthy controls. In the PD group, we also measured the plasma concentrations of the drug and of its metabolites, 3-OMD and dopamine.

The plasma homocysteine assay reagents, sodium decyl sulfate and HClO4 were purchased from Bio-Rad. l-DOPA, 3-O-methyl-dopa, dopamine, KH2PO4, and EDTA were from Sigma Chemical. Acetonitrile (HPLC grade) was purchased from Carlo Erba.

The study protocol was approved by the Institutional Ethical Committee, and written informed consent was obtained from all participants. Thirty-six PD patients who were receiving regular treatment with l-DOPA were enrolled. Patients had been previously diagnosed with idiopathic PD at the Center for Parkinson Disease and Movement Disorders of the Neurological Institute “C. Mondino” of Pavia, staged according to the criteria of Hoehn and Yahr, and evaluated with the Unified Parkinson Disease Rating Scale (18). The control group included 31 healthy subjects, matched with patients for age and sex. Subjects who had any evidence of cerebrovascular, metabolic, or endocrine disease were excluded from the study. Characteristics of the two groups are reported in Table 1.

All subjects underwent a blood sampling (10 mL), between 0900 and 1000; for PD patients, this was 2–3 h after the last dose of l-DOPA.

Blood was collected from the antecubital vein in evacuated tubes containing EDTA (Terumo Medical Corp.) and immediately refrigerated at 0–4 °C. Within 20 min, blood samples were centrifuged at 1000g for 10 min at 4 °C. The plasma obtained was separated and stored at −80 °C. All determinations were carried out within 2 weeks after the sampling.

Plasma homocysteine was determined by HPLC with fluorometric detection, using a commercially available method (Bio-Rad). Plasma samples were derivatized and subsequently deproteinized before chromatographic analysis. The HPLC system consisted of a pump equipped with a reversed-phase C18 column [70 × 3.2 mm (i.d.); 3-µm bead size; Bio-Rad]. Excitation and emission wavelengths on the fluorometric detector (Jasco) were 385 and 515 nm, respectively.

Table 1. Characteristics of PD patients and control subjects.

<table>
<thead>
<tr>
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<th>PD patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>22/14</td>
<td>19/12</td>
</tr>
<tr>
<td>Age, a years</td>
<td>62.7 ± 13.8</td>
<td>58.9 ± 7.2</td>
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<tr>
<td>Duration of the disease, a years</td>
<td>12.4 ± 5.4</td>
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<tr>
<td>L-DOPA daily intake, a mg</td>
<td>768 ± 216</td>
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<tr>
<td>Hoehn and Yahr stagea</td>
<td>2.4 ± 0.6</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>UPDRS scorea,b</td>
<td>45.6 ± 7.8</td>
<td>28.6 ± 7.3</td>
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a Mean ± SD.
b UPDRS, Unified Parkinson’s Disease Rating Scale.
Plasma 1-DOPA, 3-OMD, and dopamine were measured by HPLC with electrochemical detection (19). Briefly, for 1-DOPA and 3-OMD, plasma was deproteinized by the addition of 1.2 mol/L HClO₄ and centrifuged at 20,000g for 10 min at 4°C. The supernatant thus obtained was injected directly into the HPLC system. For dopamine, plasma samples were extracted on activated alumina before chromatographic analysis (20). The HPLC system consisted of a pump (Beckman Instruments) equipped with a reversed-phase C₁₈ column [70 × 4 mm (i.d.); 3-μm bead size; Macherey-Nagel]. The detection device was a two-electrode system (ESA Inc.), with the two electrodes set at −0.10 and +0.30 V (measuring electrode). The mobile phase (pH 2.9) consisted of 50 mmol/L KH₂PO₄, 0.7 mmol/L sodium dodecyl sulfate, and 0.3 mmol/L EDTA, mixed with 120 mL/L acetonitrile.

Signals generated from fluorometric and electrochemical detectors were collected and integrated by a dedicated personal computer, equipped with chromatography software (ValueChrom™; Bio-Rad).

We used the Student t-test for unpaired data and the Pearson correlation coefficient (r). Minimum statistical significance was set at $P < 0.05$.

Plasma homocysteine was 80% higher in PD patients than controls (mean ± SD, 16.9 ± 10.8 vs 9.3 ± 2.8 μmol/L; $P < 0.001$). To quantify the methylated catabolism of 1-DOPA, we introduced an additional variable, the 3-OMD/1-DOPA ratio. The ratio was positively correlated with homocysteine (Fig. 1). Plasma concentrations of 1-DOPA, 3-OMD, and dopamine in PD patients were 1.7 ± 1.2 mg/L, 19.7 ± 12 mg/L, and 0.7 ± 0.6 μg/L, respectively. We found no significant correlations between homocysteine and the concentrations of 1-DOPA or its metabolites. Analogously, no correlations were observed between homocysteine concentrations and the daily intake of the drug or the scores of PD severity.

High concentrations of blood homocysteine currently are considered a prothrombotic condition and, more in general, an independent risk factor for cardiovascular diseases (3–5). Recent evidence has shown that homocysteine may also play a role in the pathogenesis of neurodegenerative disorders (7–9). Indeed, various studies have shown increased concentrations of plasma homocysteine in patients with PD (14–17). The increase, however, is observable in patients under treatment with 1-DOPA, whereas untreated PD patients have homocysteine concentrations within the reference interval. Therefore, it has been suggested that the drug, rather than the disease per se, plays a major role in the hyperhomocysteinemia of PD patients (17).

In our study, we confirmed that PD patients treated with 1-DOPA have higher plasma homocysteine than control subjects. Both the magnitude of the increase found in the patients and the absolute values of homocysteine in the two groups were similar to the values reported by other groups (14–17), thus showing a substantial reproducibility of the phenomenon.

We observed no significant correlations between homocysteine and either the plasma concentrations of 1-DOPA, 3-OMD, and dopamine or the daily intake of the drug. This may seem difficult to reconcile with a direct role of 1-DOPA in the hyperhomocysteinemia of PD patients. Recently, Yasui et al. (21) reported increased plasma homocysteine in PD patients treated with 1-DOPA, but similar to our study, they found no correlation with the daily intake of the drug. However, the authors showed that homocysteine was further increased in a subpopulation of PD patients homozygous for a mutation (C677T) in the gene encoding for the enzyme 5,10-methylenetetrahydrofolate reductase, whose deficiency causes hyperhomocysteinemia (11). Conversely, controls subjects with the same mutation did not differ, in terms of plasma homocysteine, from the rest of the control population. It follows that 1-DOPA administration alone may not be sufficient to cause hyperhomocysteinemia unless the therapy is associated with an enzymatic defect in the remethylation pathway of the amino acid. Indeed, we found a positive correlation between homocysteine and the 3-OMD/1-DOPA ratio, an additional variable that we introduced as an index of the methylated catabolism of 1-DOPA. This would further support the hypothesis that the processes of methyl-group transfer involved in the transformation of 1-DOPA into 3-OMD may interfere with the metabolism of homocysteine, causing accumulation of the amino acid. Further investigation, however, will be required to establish a clear cause–effect relationship between 1-DOPA therapy and hyperhomocysteinemia.

It is well known that oxidative stress plays a major role in the neurodegenerative process that underlies PD (22). Various experimental studies have also shown that 1-DOPA may paradoxically contribute to neuronal damage through the formation of free radicals (23–25). Indeed, we recently reported increased formation of hydroxyl radicals in blood cells of PD patients under treatment with 1-DOPA, compared with both untreated PD patients and
healthy subjects (26). Because the adverse effects of homocysteine are most likely related to its prooxidant properties (6), a direct involvement of the amino acid in this phenomenon may be therefore hypothesized.

In conclusion, PD patients undergoing regular treatment with L-DOPA have higher plasma homocysteine concentrations than healthy subjects. The increase seems to be related to the methylated catabolism of the drug, although other factors, such as enzymatic defects in the remethylation pathway of homocysteine, are likely to play a substantial role. Increased risk of cerebro- and cardiovascular diseases has been reported in PD patient populations, although the issue is highly disputed (27, 28). Whether the increase in plasma homocysteine occurring in PD patients plays a role in the progression of the disease remains to be established.

This study was supported by a grant of the Ministry of Health of the Italian Government to the Neurological Institute C. Mondino (Ricerca Corrente 1998).

References

5. Perry U, Refsum H, Morris RW, Ebrahim B, Ueland PM. Increased risk of cerebro- and cardiovascular diseases has been reported in PD patient populations, although the issue is highly disputed (27, 28). Whether the increase in plasma homocysteine occurring in PD patients plays a role in the progression of the disease remains to be established.

Enzymes of the cytochrome P450 system are involved in the metabolism of a broad range of foreign compounds, such as drugs, environmental pollutants, and carcinogens (1). The most abundant enzyme in the human liver is cytochrome P450 3A4 (CYP3A4) (2). This enzyme is involved in the metabolism of >50% of all drugs used in humans (3, 4), and the interindividual differences in the pharmacokinetics of these drugs are thought to be related to variations in CYP3A4 activity (4–6). These variations may be caused by age and disease-related differences, by drugs inducing or repressing transcription/translation, or by genetic polymorphisms. Although the CYP3A4 gene was initially thought not to be polymorphic, recent reports have described three genetic variants of this gene: CYP3A4*1B, CYP3A4*2, and CYP3A4*3 (7, 8). The allelic frequency for the CYP3A4*1B allele, which contains an A→G substitution in the promoter region of CYP3A4, ranges from 0.0% in Chinese and Japanese Americans to >54% in African Americans (8, 9). American and European Caucasians were reported to have an allelic frequency of ~4–5% (8–11). The CYP3A4*2 allele, which encodes a Ser222Pro change, has an allelic frequency of 2.7% in the white (Finnish) population (8). Because variant alleles that are found in >1% of the population are defined as genetic polymorphisms (12), both the CYP3A4*1B and the CYP3A4*2 allele are consid-