Elevated Plasma Homocysteine Levels in Patients Treated With Levodopa

John D. Rogers, MD; Anna Sanchez-Saffon, BA; Alan B. Frol, PhD; Ramon Diaz-Arrastia, MD, PhD

Background: Hyperhomocysteinemia is a risk factor for vascular disease and potentially for dementia and depression. The most common cause of elevated homocysteine levels is deficiency of folate or vitamin B\textsubscript{12}. However, patients with Parkinson disease (PD) may have elevated homocysteine levels resulting from methylation of levodopa and dopamine by catechol O-methyltransferase, an enzyme that uses S-adenosylmethionine as a methyl donor and yields S-adenosylhomocysteine. Since S-adenosylhomocysteine is rapidly converted to homocysteine, levodopa therapy may put patients at increased risk for vascular disease by raising homocysteine levels.

Objectives: To determine whether elevations in plasma homocysteine levels caused by levodopa use are associated with increased prevalence of coronary artery disease (CAD), and to determine what role folate and vitamin B\textsubscript{12} have in levodopa-induced hyperhomocysteinemia.

Design/Methods: Subjects included 235 patients with PD followed up in a movement disorders clinic. Of these, 201 had been treated with levodopa, and 34 had not. Blood samples were collected for the measurement of homocysteine, folate, cobalamin, and methylmalonic acid levels. A history of CAD (prior myocardial infarctions, coronary artery bypass grafting, or coronary angioplasty procedures) was prospectively elicited. We analyzed parametric data by means of 1-way analysis of variance or the t test, and categorical data by means of the Fisher exact test or \( \chi^2 \) test.

Results: Mean ± SD plasma homocysteine levels were significantly higher in patients treated with levodopa (16.1 ± 6.2 µmol/L), compared with levodopa-naïve patients (12.2 ± 4.2 µmol/L; \( P = .001 \)). We found no difference in the plasma concentration of folate, cobalamin, or methylmalonic acid between the 2 groups. Patients whose homocysteine levels were in the higher quartile (≥ 17.7 µmol/L) had increased prevalence of CAD (relative risk, 1.75; 95% confidence interval, 1.08-2.70; \( P = .04 \)).

Conclusions: Levodopa therapy, rather than PD, is a cause of hyperhomocysteinemia in patients with PD. Deficiency of folate or vitamin B\textsubscript{12} levels does not explain the elevated homocysteine levels in these patients. To our knowledge, this is the first report that levodopa-related hyperhomocysteinemia is associated with increased risk for CAD. These findings have implications for the treatment of PD in patients at risk for vascular disease, and potentially for those at risk for dementia and depression.

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among hyperhomocysteinemia, levodopa therapy, and vitamin levels has not been clearly established. Prior studies have examined European patients, populations that have not been exposed to vitamin supplementation of foodstuffs, which was mandated by the US Food and Drug Administration starting in January 1998. The effect of over-the-counter vitamin supplements was collected. Information regarding the presence of vascular disease in patients with PD are within the range that predicts increased risk for CAD and stroke, prior studies have not examined the prevalence of vascular disease in patients with PD and hyperhomocysteinemia. We undertook this study to determine whether elevated plasma homocysteine levels in patients with PD are associated with CAD, and to elucidate the possible effects that folate and vitamin B12 may have on homocysteine metabolism in this population.

**METHODS**

Subjects were selected from a patient registry of PD. The patients were followed up in a specialty clinic in New York, NY, from 1993 through 2001. The registry consisted of 411 subjects, and plasma homocysteine values were obtained from 239. Of these, 34 (14.2%) had never been treated with levodopa (21 were treated with other dopaminergic agonists, and 13 had not received any dopaminergic therapy), and 205 (85.8%) were receiving levodopa therapy. Information regarding age, sex, ethnicity, therapy, duration of illness, and use of multivitamin supplements was collected. Information regarding the presence of CAD (prior myocardial infarctions, coronary artery bypass grafting, or coronary angioplasty procedures) was obtained through patient interview and review of hospital records at the time that homocysteine levels were measured.

Nonfasting blood samples were drawn from a peripheral vein and immediately refrigerated. Plasma homocysteine levels were determined by means of a fluorescence polarization immunoassay, using the Imx instrument (Abbott Laboratories, Chicago, Ill). Intraindividual variations of plasma homocysteine levels measured under these conditions are small, with coefficients of variation of less than 10%. We determined folate and vitamin B12 levels by means of immunoassay, and methylmalonic acid levels by means of gas chromatography/mass spectroscopy. We analyzed parametric data by means of 1-way analysis of variance (ANOVA) or 2-tailed t test, and categorical data by means of the Fisher exact test or the \( \chi^2 \) test. Multiple linear regression analysis examined the combined contribution of age, duration of levodopa treatment, and levels of folate and vitamin B12 to homocysteine levels.

Since the distribution of homocysteine values was positively skewed, a common logarithm transformation was used in statistical tests to better meet the assumption of normality. Statistical analysis was performed using the InStat program, version 3.0 (GraphPad Software, San Diego, Calif), or SPSS, version 10.1 (SPSS Inc, Chicago, Ill).

**RESULTS**

As anticipated, patients treated with levodopa were older and had had a diagnosis of PD for approximately 4 years longer than patients who had not received levodopa (Table 1). The mean duration of levodopa treatment was slightly more than 5 years. Mean plasma homocysteine levels were 31% higher in levodopa-treated patients \( (P < .001) \) (Table 1). Since increased age and male sex are independently associated with higher homocysteine levels, homocysteine levels were adjusted for age and sex. After adjustment, the differences in homocysteine levels remained highly significant between the 2 groups (Table 1). We found no differences in mean serum cobalamin or folate levels between the 2 groups. Furthermore, we did not find that serum methylmalonic acid levels correlated with plasma homocysteine levels.

Plasma homocysteine concentrations in levodopa-treated patients did not follow a Gaussian distribution. Figure 1 shows that the curve of levodopa-treated patients is shifted to the right by approximately 3.7 \( \mu \)mol/L. In addition, a subgroup of levodopa-treated patients, constituting 20% of the cohort, had homocysteine levels of at least 20 \( \mu \)mol/L. Only 2 (6%) levodopa-naïve patients had similarly elevated homocysteine levels (odds ratio, 4.0; \( P = .05 \)).

To further analyze the effects of vitamin concentrations on plasma homocysteine levels, we stratified homocysteine levels into quartiles (Table 2). One-way ANOVA showed significant differences between quartiles for age, duration of illness, and levels of vitamin B12 and folate. Age at diagnosis showed a nonsignificant trend (\( P = .60 \)). These

**Table 1. Effect of Levodopa Therapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Levodopa Therapy (n = 34)</th>
<th>Levodopa Therapy (n = 205)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.2 ± 13.6</td>
<td>72.7 ± 10.6</td>
<td>.003†</td>
</tr>
<tr>
<td>% Male</td>
<td>50</td>
<td>61</td>
<td>.26</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>59.1 ± 12.5</td>
<td>63.4 ± 12.2</td>
<td>.08</td>
</tr>
<tr>
<td>Duration of illness, y</td>
<td>4.8 ± 1.9</td>
<td>8.9 ± 5.3</td>
<td>.003†</td>
</tr>
<tr>
<td>Duration of treatment with levodopa, y</td>
<td>...</td>
<td>5.5 ± 4.4</td>
<td>.003†</td>
</tr>
<tr>
<td>Total homocysteine level, ( \mu )mol/L</td>
<td>12.2 ± 4.2</td>
<td>16.1 ± 6.2</td>
<td>&lt;.001‡</td>
</tr>
<tr>
<td>Age- and sex-corrected total homocysteine level, ( \mu )mol/L</td>
<td>14.4 ± 2.0</td>
<td>15.7 ± 1.9</td>
<td>.001†</td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td>18.7 ± 2.7</td>
<td>17.7 ± 6.1</td>
<td>.35</td>
</tr>
<tr>
<td>Vitamin B12, pg/mL</td>
<td>564.8 ± 293.9</td>
<td>566.5 ± 378.0</td>
<td>.98</td>
</tr>
<tr>
<td>Methylmalonic acid, ( \mu )mol/L</td>
<td>321.1 ± 212.3</td>
<td>383.0 ± 270.7</td>
<td>.35</td>
</tr>
</tbody>
</table>

*†Significant.*

**Figure 1.** Cumulative distribution of plasma homocysteine levels of levodopa-treated patients and patients who did not receive levodopa.
Multiple reports indicate that plasma homocysteine concentrations of greater than 14 µmol/L are associated with approximately a doubling of the risk for vascular disease, including CAD. Since half of our patients had homocysteine elevations to this level, we determined whether levodopa-associated hyperhomocysteinemia, which may result from a different mechanism than hyperhomocysteinemia in the general population, was associated with increased risk for CAD. The overall prevalence of CAD in this cohort of elderly patients was high (33%). Patients in the highest homocysteine quartile (≥17.7 µmol/L) had a 1.7-fold relative risk for CAD, compared with the other three quartiles (95% confidence interval, 1.08-2.70; P = .04) (Figure 2). We did not find an increased risk for CAD in patients with moderate levodopa-associated homocysteine elevations ranging from 14.1 to 17.6 µmol/L, levels that are associated with CAD in other populations. Our data did not allow us to determine the duration or severity of CAD in our cohort.

Homocysteine has received substantial attention for the past 10 years as an important and potentially readily reversible risk factor for vascular disease, including CAD, and possible risk factors for neurodegenerative disease. Although the mechanism of this association is not well understood, a possible mechanism is through the decrease in blood levels of folate and vitamin B12 that occurs in conditions associated with increased homocysteine levels. The results suggest that older age, longer duration of PD or levodopa use, and lower levels of vitamin B12 and folate are risk factors for hyperhomocysteinemia. Since most subjects not treated with levodopa were in the lowest quartile of homocysteine concentration, there was a difference in levodopa use between the upper quartile and the lowest quartiles. However, we found no difference when the highest quartile was compared with the other three quartiles. Age, duration of levodopa treatment, and vitamin B12 level were significant contributors to homocysteine concentration in multiple regression analysis, whereas the contribution of folate level was nearly significant.

Since low levels of folate and cobalamin are the most common causes of hyperhomocysteinemia in the general population, we studied the relationship between vitamin and homocysteine levels in our population of patients with PD. No patients met the standard definition of folate deficiency (<2.6 ng/mL [<5.9 nmol/L]), and only 3 patients (1 in the lowest homocysteine-level quartile and 2 in the highest quartile) met the standard definition of cobalamin deficiency (<211 pg/mL). However, although all quartiles had mean folate and vitamin B12 levels well within the reference range, subjects with the highest plasma homocysteine levels had significantly lower mean blood levels of both vitamins (Table 2). The high mean vitamin levels may be partly due to the finding that most patients used over-the-counter multivitamin supplements. Use of vitamin supplements did not correlate with homocysteine levels in our population. Subjects used a variety of different multivitamin preparations, and we were unable to determine dosages of vitamin supplements used.

Table 2. Analysis by Homocysteine-Level Quartiles*

<table>
<thead>
<tr>
<th>Homocysteine level, µmol/L</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>9.4 ± 1.3</td>
<td>12.7 ± 0.8</td>
<td>15.8 ± 1.1</td>
<td>23.7 ± 6.0</td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>6.0-11.2</td>
<td>11.3-14.0</td>
<td>14.1-17.6</td>
<td>17.7-46.7</td>
<td>NA</td>
</tr>
<tr>
<td>Age, y</td>
<td>66.9 ± 12.5</td>
<td>71.6 ± 12.2</td>
<td>72.4 ± 11.5</td>
<td>75.6 ± 9.2</td>
<td>&lt;.001‡</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>59.8 ± 12.5</td>
<td>64.6 ± 12.4</td>
<td>62.9 ± 12.3</td>
<td>64.8 ± 11.4</td>
<td>.17</td>
</tr>
<tr>
<td>Duration of illness, y</td>
<td>6.8 ± 4.6</td>
<td>7.4 ± 4.8</td>
<td>8.9 ± 5.0</td>
<td>9.7 ± 6.0</td>
<td>.03‡</td>
</tr>
<tr>
<td>Levodopa therapy, %</td>
<td>29</td>
<td>87</td>
<td>92</td>
<td>94</td>
<td>&lt;.001‡</td>
</tr>
<tr>
<td>Duration of therapy, y</td>
<td>2.9 ± 3.3</td>
<td>4.4 ± 4.1</td>
<td>5.7 ± 5.3</td>
<td>6.1 ± 4.8</td>
<td>.02‡</td>
</tr>
<tr>
<td>Folate level, ng/mL</td>
<td>18.8 ± 3.3</td>
<td>17.9 ± 3.8</td>
<td>18.7 ± 8.1</td>
<td>15.8 ± 4.7</td>
<td>&lt;.001‡</td>
</tr>
<tr>
<td>Vitamin B12 level, pg/mL</td>
<td>703.4 ± 483.1</td>
<td>523.8 ± 242.1</td>
<td>503.8 ± 299.3</td>
<td>475.2 ± 243.0</td>
<td>.01‡</td>
</tr>
<tr>
<td>Methylenonic acid, µmol/L</td>
<td>344.2 ± 302.6</td>
<td>228.2 ± 127.9</td>
<td>437.2 ± 230.8</td>
<td>432.6 ± 280.4</td>
<td>.16</td>
</tr>
<tr>
<td>Multivitamin use, %</td>
<td>75</td>
<td>80</td>
<td>68</td>
<td>62</td>
<td>.19</td>
</tr>
<tr>
<td>Patients with CAD, % of known</td>
<td>30</td>
<td>22</td>
<td>32</td>
<td>48</td>
<td>.04†</td>
</tr>
<tr>
<td>Relative risk for CAD (95% CI)</td>
<td>0.874 (0.507-1.507)</td>
<td>0.627 (0.312-1.259)</td>
<td>0.938 (0.547-1.611)</td>
<td>1.705 (1.075-2.703)</td>
<td>.04‡</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; CI, confidence interval; NA, not applicable.

*Data are given as mean ± SD unless otherwise indicated.
†Based on comparisons for quartile 4 vs quartiles 1 through 3.
‡Considered significant.

Figure 2. Relative risk for coronary artery disease (CAD) by quartile of homocysteine levels. The fraction of patients with any history of CAD (prior myocardial infarction, coronary artery bypass grafting, or coronary angioplasty procedures) was calculated for each quartile of plasma homocysteine distribution. We calculated relative risk (with 95% confidence intervals) by comparison with the other three quartiles, using the Fisher exact test.
Several groups have analyzed plasma homocysteine levels in patients treated with levodopa. Allain et al\(^\text{16}\) found that patients with PD had mean homocysteine levels 48% higher than controls, but the controls in their study did not have PD, making it impossible to distinguish the effects of therapy from factors related to the underlying disease. It is also unclear whether the control group in their study was matched for age with the PD group. Kuhn et al\(^\text{17}\) repeated these findings, but the age-matched control group in their study also did not have PD. Muller et al\(^\text{18}\) confirmed these observations\(^{16,18}\) and for the first time used a group of nontreated patients with PD as an age- and sex-matched control group, indicating that therapy, rather than PD, was the cause of hyperhomocysteinemia. However, no information was provided regarding duration of illness, vitamin levels, or prevalence of vascular disease in their report. Yasui et al\(^\text{19}\) confirmed these findings\(^{16-18}\) in a Japanese population, and for the first time analyzed folate and vitamin B\(_2\) levels. They found that mean vitamin levels in patients with PD were in the reference range, but did not report on vitamin levels in the non-parkinsonian control group. This study\(^\text{19}\) found that inheritance of the T/T MTHFR genotype resulted in significantly higher plasma homocysteine levels in levodopa-treated patients.

Potentially the most interesting result of our study is the finding that in patients treated with levodopa, homocysteine elevations are associated with an increased risk for CAD. Although the relative risk is modest (1.7-fold), the prevalence of CAD is high in this elderly population; thus, the magnitude of the homocysteine-associated risk is substantial. It is likely that other factors, such as age and relatively low levels of folate and vitamin B\(_2\), in combination with levodopa use, contributed to homocysteine elevations. However, we believe that levodopa therapy was the predominant factor causing hyperhomocysteinemia. First, we did not find comparably high homocysteine levels in patients with PD who were not treated with levodopa, even after adjusting for age differences between the 2 groups. Second, others have also shown that levodopa use is associated with elevated homocysteine levels, regardless of age.\(^{17,19}\) Finally, experience in animal models\(^{18}\) indicates that levodopa therapy results in elevations of plasma homocysteine levels. These results support the hypothesis that excessive production of SAH and/or exhaustion of methyl groups, as a result of COMT-mediated methylation of levodopa and dopamine, are the causative mechanism of hyperhomocysteinemia in levodopa-treated patients with PD.

The cross-sectional nature of our study also does not allow us to determine the duration of levodopa-associated hyperhomocysteinemia that results in increased CAD risk. Our data indicate that patients in the highest homocysteine-level quartile had received treatment with levodopa for more than 6 years; however, the duration of therapy was comparably long in patients in the second and third quartiles of the homocysteine distribution, who did not have increased CAD risk.

In addition to CAD, patients with hyperhomocysteinemia are at increased risk for venous thrombosis, peripheral vascular disease, and stroke.\(^{4,39,50}\) Among stroke
Our findings in a North American cohort confirm the results of European and Japanese studies that indicate that levodopa therapy results in elevated homocysteine levels. Folate and vitamin B12 levels are within the normal range in levodopa-treated hyperhomocysteinemic patients, but supraphysiological vitamin levels may confer some protection. Finally, we report that the time that elevated homocysteine levels in the setting of levodopa use are associated with an increased risk for CAD. These observations have important implications in the treatment of patients with PD who are at risk for vascular disease, and potentially for those at risk for dementia and depression.

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Author contributions: Study concept and design (Drs Rogers and Diaz-Arrastia and Ms Sanchez-Saffon); acquisition of data (Drs Rogers and Frol and Ms Sanchez-Saffon); analysis and interpretation of data (Drs Rogers, Frol, and Diaz-Arrastia); drafting of the manuscript (Dr Diaz-Arrastia); critical revision of the manuscript for important and intellectual content (Drs Rogers, Frol, and Diaz-Arrastia and Ms Sanchez-Saffon); statistical expertise (Drs Frol and Diaz-Arrastia); obtained funding (Dr Diaz-Arrastia); administrative, technical, and material support (Drs Rogers and Ms Sanchez-Saffon); study supervision (Dr Diaz-Arrastia).

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REFERENCES


