Elevated Plasma Homocysteine Levels in Patients Treated With Levodopa

Association With Vascular Disease

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 B12. 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 B12 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) or affective disorders.14,15Elevations in 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 B12 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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From the Yarmen Center for Parkinson’s Disease, Department of Neurology, Beth Israel Medical Center, New York, NY (Dr Rogers and Ms Sanchez-Saffon); and the Division of Neuropsychology, Departments of Psychiatry (Dr Frol) and Neurology (Dr Diaz-Arrastia), The University of Texas Southwestern Medical Center, Dallas.

Elevations in plasma levels of homocysteine, a sulfur-containing amino acid, is an emerging risk factor for vascular disease, including coronary artery disease (CAD), stroke, peripheral vascular disease, and venous thrombosis.1-6 More recently, hyperhomocysteinemia has been implicated as a risk factor in Alzheimer disease7-11 and has been associated with mild cognitive deficits in nondemented elderly subjects12,13 and with affective disorders.14,15 Elevations in plasma homocysteine levels result from a complex interaction of acquired and genetic factors, but quantitatively the most important are deficiencies of folate, vitamin B12, and vitamin B6.1 Recently, several reports16-10 have indicated that patients with Parkinson disease (PD) have significant elevations of plasma homocysteine levels. In PD, elevated homocysteine levels may result from treatment with levodopa, rather than vitamin deficiency. An important pathway for metabolism of levodopa is methylation by catechol O-methyltransferase (COMT), an enzyme that uses S-adenosylmethionine as a methyl donor and yields S-adenosylhomocysteine (SAH). Since SAH is rapidly converted to homocysteine, levodopa therapy may put patients at increased risk for vascular disease by raising homocysteine levels.

Although several groups have reported elevated plasma homocysteine levels in patients with PD, the relationship...
among hyperhomocysteinemia, levodopa therapy, and vitamin levels has not been clearly established. Prior studies have examined European \cite{16-18} and Japanese \cite{19} 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.\footnote{The effect of over-the-counter vitamin supplements was mandated by means of immunoassay, and methylmalonic acid levels by 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 \chi² test. Multiple linear regression analysis examined the combined contribution of age, duration of levodopa treatment, and levels of folate and vitamin \( B_{12} \) 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).}

The effect of over-the-counter vitamin supplements in PD-associated hyperhomocysteinemia has likewise not been studied. Furthermore, although the elevations of homocysteine levels found in patients with PD are within the range that predicts increased risk for CAD and stroke,\cite{15} 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 \( B_{12} \) 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 interviews 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%.\footnote{We determined folate and vitamin \( B_{12} \) levels by means of immunoassay, and methylmalonic acid levels by means of gas chromatography/mass spectroscopy.}

## 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 µmol/L. In addition, a subgroup of levodopa-treated patients, constituting 20% of the cohort, had homocysteine levels of at least 20 µmol/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 \( B_{12} \) and folate. Age at diagnosis showed a nonsignificant trend (\( P = .60 \)). These
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 3 lowest quartiles. However, we found no difference when the highest quartile was compared with the 2 middle 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 cobalamin deficiency (<2.6 ng/mL [<5.9 mmol/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. Patients used a variety of different multivitamin preparations, and we were unable to determine dosages of vitamin supplements used.

Multiple reports⁴ 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 3 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.

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.

SI conversion factors: To convert folate to nanomoles per liter, multiply by 2.266; and vitamin B₁₂ to picomoles per liter, multiply by 0.738.

ªBased 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 3 other quartiles, using the Fisher exact test.
peripheral arterial disease, and stroke. A meta-analysis of 27 studies concluded that 10% of the risk for CAD was caused by elevation of homocysteine levels, and that a 5-mmol/l increase in plasma homocysteine level was comparable to a 20-mg/dl (0.5-mmol/l) increase in serum cholesterol level. These observations have prompted several large randomized controlled trials that are currently under way to determine whether vitamin therapy to lower homocysteine levels decreases the risk for vascular disease. One recently published secondary prevention trial found that therapy that lowered homocysteine levels significantly decreased the risk for restenosis of coronary arteries and improved clinical outcome after coronary angioplasty procedures. Elevated homocysteine levels have also been implicated as a risk factor for Alzheimer disease, depression, and cognitive deficits in nondemented elderly subjects. Thus, therapy that results in increased homocysteine levels has the potential to put patients at increased risk for vascular disease and other neuropsychiatric disorders.

In the general population, the most common causes of elevated homocysteine levels are deficiencies in folic acid, vitamin B6, and vitamin B12. Data from the Framingham Study indicate that inadequate plasma concentrations of one or more of these B vitamins contributes to 67% of cases of elevated homocysteine levels. Old age, male sex, and other acquired factors are also common causes of hyperhomocysteinemia. Genetic factors, including common single-nucleotide polymorphisms in methylenetetrahydrofolate reductase (C677T MTHFR polymorphism), methionine synthase (A2556G polymorphism), and cystathionine β-synthase (T833C CBS polymorphism), may also result in elevated homocysteine levels in some patients.

An important acquired cause of hyperhomocysteinemia is levodopa therapy. Levodopa and dopamine are partly metabolized by methylation, a reaction catalyzed by COMT. This enzyme uses S-adenosylmethionine as the methyl donor producing SAH, which is subsequently converted to homocysteine by SAH hydrolase. Homocysteine can be remethylated to methionine by methionine synthase, a reaction that uses methyltetrahydrofolate as the methyl donor and cobalamin as a cofactor. Homocysteine can also be eliminated through the urine after metabolism by the transulfuration pathway, through condensation with serine to form cystathionine. This reaction is catalyzed by cystathionine β-synthase and uses pyridoxal phosphate as a cofactor. Thus, in the setting of levodopa use, elevations of homocysteine levels can result from the following 3 mechanisms, which are not mutually exclusive and can coexist in any given patient: (1) excess production of SAH by COMT; (2) decrease in remethylation of homocysteine, which may result from exhaustion of the pool of methyl groups, relative deficiency of folic acid and vitamin B12 levels, or genetically determined inefficiency of methionine synthase or methylenetetrahydrofolate reductase; and (3) deficits in the transulfuration pathway, which may result from deficiency of vitamin B6 or genetic inefficiency of cystathionine β-synthase. Knowledge of the pathway(s) that is important in producing hyperhomocysteinemia during levodopa therapy is an important step in developing strategies to prevent it.

Several groups have analyzed plasma homocysteine levels in patients treated with levodopa. Allain et al 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 examined these findings, but the age-matched control group in their study also did not have PD. Muller et al confirmed these observations, and the first time used a group of non-treated 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 confirmed these findings in a Japanese population, and for the first time analyzed folate and vitamin B12 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 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 folic acid and vitamin B12, 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. Finally, experience in animal models 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. Among stroke
mechanisms, hyperhomocysteinemia is most closely associated with subcortical microvascular infarctions,\textsuperscript{41,42} an important problem in PD because subcortical infarctions may produce or exacerbate parkinsonian symptoms. Our data do not allow us to determine whether levodopa-associated hyperhomocysteinemia was associated with cerebral microinfarctions, an important issue that needs further study. More recently, elevated homocysteine levels have been identified as risk factors for dementia,\textsuperscript{6-10} cognitive slowing,\textsuperscript{12,13} and depression.\textsuperscript{15} Dementia\textsuperscript{3} or affective symptoms\textsuperscript{2} develop in a significant fraction of patients with PD, but our dataset does not allow us to correlate these neuropsychiatric symptoms with elevations of homocysteine levels. Establishment of such a link will require prospective collection of neuropsychological data on a large cohort of patients with PD.

Almost all of our patients had normal to high folate and vitamin B\textsubscript{12} levels. The high folate levels may result from supplementation of cereals, grains, and other foodstuffs in the United States, as well as the high fraction of our subjects who were using over-the-counter multivitamin preparations. Only a miniscule fraction of our patients were deficient in folate or vitamin B\textsubscript{12}, by the reference standard. Mean levels of folate and vitamin B\textsubscript{12} for patients in the highest homocysteine quartile were substantially higher than those associated with comparable homocysteine elevations in patients who did not use levodopa. Selhub et al,\textsuperscript{1} studying the Framingham cohort, did not find additional lowering of homocysteine levels as an effect of folate levels of greater than 4.4 ng/mL (\textgtr 10 nmol/L) or of vitamin B\textsubscript{12} levels of greater than 348 pg/mL (>257 pmol/L), whereas vitamin concentrations for subjects in the highest homocysteine quartile were higher by a significant margin (mean ± SD, 15.8 ± 4.7 ng/mL [35.9 ± 10.7 nmol/L] and 475.2 ± 243.0 pg/mL [350.7 ± 179.3 pmol/L], respectively; Table 2). However, patients with PD with the highest homocysteine concentrations had marginally, but significantly, lower folate and vitamin B\textsubscript{12} levels than those in the 3 lower quartiles. These results suggest that levodopa treatment increases the risk of these vitamins, and raises the possibility that supplementation with high doses of folic acid and cobalamin may prevent levodopa-induced hyperhomocysteinemia. Alternatively, it is possible that treatment with COMT inhibitors or supplementation of the diet with S-adenosylmethionine (to replenish stores of methyl groups) may be required to lower homocysteine levels in levodopa-treated patients. Addressing these important issues will likely require a randomized, prospective trial.

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 B\textsubscript{12} levels are within the normal range in levodopa-treated hyperhomocysteinemic patients, but supraphysiological vitamin levels may confer some protection. Finally, we report for the first 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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Corresponding author and reprints: Ramon Diaz-Arrastia, MD, PhD, Department of Neurology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9036 (e-mail: Ramon.Diaz-Arrastia@UTSouthwestern.edu).

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