Levodopa-Associated Increase of Homocysteine Levels and Sural Axonal Neurodegeneration

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Background: Levodopa metabolism via catechol O-methyltransferase increases levels of the neurotoxin homocysteine, which induces an axonal-accentuated degeneration in sensory peripheral nerves in vitro.

Objectives: To demonstrate associations among daily levodopa/dopa decarboxylase inhibitor intake, total homocysteine plasma (tHcy) levels, and electrophysiologic sural nerve conduction findings.

Design: We performed bilateral assessment of sensory nerve conduction velocity and sensory nerve action potentials and determined tHcy levels.

Patients: Thirty-one levodopa-treated patients with Parkinson disease (PD) and 27 control subjects.

Results: Sensory nerve action potentials significantly (P < .001) differed between PD patients and controls. No differences between sensory nerve conduction velocities of PD patients and controls appeared. We found significant differences in sensory nerve action potentials between PD patients with significantly elevated tHcy levels and controls (P < .001), PD patients with tHcy levels within the reference range and those with elevated levels (P = .001), and PD patients with tHcy levels levels within the reference range and and controls (P = .04). Our sensory nerve conduction velocity results showed no significant differences. There were significant associations between tHcy levels and sensory nerve action potentials (R = −0.52; P = .002) and and sensory nerve conduction velocity (R = −0.47; P = .008). Daily levodopa/dopa decarboxylase inhibitor intake was significantly related to tHcy levels (R = 0.43; P = .02).

Conclusions: This electrophysiological sign of peripheral neuronal dysfunction may be circumstantial evidence suggesting that, to a certain extent, sensory nerve action potentials are a surrogate marker for the levodopa metabolism–induced elevation of homocysteine levels and the aggravation of the ongoing central neurodegenerative process.

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lism, which is associated with long-term levodopa/DDI application, is neurotoxic in PD patients. However, increased tHcy levels contribute to the incidence of vascular disease, brain atrophy, Alzheimer disease, or all of these. A sensitive, clinical, biological surrogate neurotoxin marker could be a primary axonal-accentuated degeneration with secondary demyelination of sural nerves. The objective of the present study was to demonstrate an association between daily oral levodopa/DDI intake, tHcy plasma levels, and electrophysiologic sural nerve conduction findings.

METHODS

SUBJECTS

We enrolled 31 PD patients undergoing long-term levodopa/DDI treatment and 27 control subjects into this trial (Table 1). Before enrollment, we excluded individuals with metabolic disturbances, (eg, diabetes), abnormal in vivo levels of vitamins (ie, vitamins B1, B6, and B12 and folate) and methylmalonic acid, neurotoxicity, (eg, diabetes), abnormal in vivo levels of vitamins (ie, vitamins B1, B6, and B12 and folate) and methylmalonic acid, neurotoxicity, metabolic disturbances, and neoplasms.

Each patient fasted and did not take any antiparkinsonian medication for at least 12 hours before blood samples were drawn. The samples were then centrifuged, decanted, and stored at -80°C. The period from freezing to workup of the plasma samples was no longer than 3 months. We measured levels of tHcy by means of automated high-performance liquid chromatography with reverse-phase separation and fluorometric detection, with sodium borohydride/monobromobimane (NaBH4/mBrB) reduction followed by monobromobimane derivatization. We performed bilateral sural nerve conduction assessment in all PD patients after obtaining blood specimens and in all controls using an established, standardized technique (electromyograph, Dantec Keypoint, Skovlunde, Denmark; skin temperature, 34°C; average of at least 20 sensory nerve action potentials [SNAPs]; supramaximal stimulation; and surface recording). Data of sensory nerve conduction velocity (SCV) and SNAPs represent the sum of the right and left sides (Table 1).

We also performed a semistructured open interview about signs of neuropathy in the enrolled PD patients. We asked about the onset of cramps in the legs during the “on” periods of medication, if applicable. We scored leg cramps on a scale where 0 indicates never; 1, seldom; 2, frequent; and 3, daily. A standardized set of questions asked about sensitive symptoms (eg, dysesthesia, paresthesia, pain, and burning) in the “on” periods, if applicable. We rated sensitive symptoms on a scale where 0 indicates no; 1, light; 2, severe; and 3, strong complaints.

We investigated reflexes of the lower limbs, ie, Achilles tendon reflex and patellar reflex, in the supine position. Lower limb reflexes were evaluated on a scale where 0 indicates present; 1, intensity of the Achilles tendon reflex is less than the patellar reflex; 2, no Achilles tendon reflex; and 3, no Achilles tendon reflex and no patellar reflex. Clinical evaluation of onset of leg cramps, sensitive symptoms, and reflexes of the lower limb outcomes were added for the statistical analysis.

We measured the perception of vibration with a tuning fork at both medial malleoli. Results were evaluated on a scale in which 0 indicates no and 8, optimum perception. We added results of the right and left sides for statistical calculations.

ETHICS

Each subject gave informed consent. The local ethics committee of Ruhr University Bochum, Bochum, Germany, approved this study.

STATISTICS

We used analysis of covariance and set age and sex as covariates for comparisons. We used the Tukey Honestly Significant Difference test for different numbers for the post hoc analysis. We divided PD patients according to their tHcy plasma levels at 15 µmol/L or less (reference range) and greater than 15 µmol/L (elevated tHcy levels). We used linear regression for correlation analysis. The level of significance was P<.05.

RESULTS

The tHcy level was significantly higher in the PD patients with elevated tHcy levels compared with PD patients with tHcy levels within the reference range (“reference tHcy levels”) and controls, both of whom did not differ from one another in the post hoc analysis (Table 1 and Table 2). The SNAPs were significantly lower in PD patients than

| Table 1. Clinical Characteristics of Study Patients and Controls |
|------------------|------------------|------------------|------------------|
| **PD (n = 31)**  | **PD With Reference tHcy Values (n = 11)** | **PD With Elevated tHcy Values (n = 20)** | **Controls (n = 27)** |
| Age, y           | 62.65 ± 7.3 (48-79) | 61.36 ± 4.67 (56-71) | 63.35 ± 8.43 (48-79) | 59.56 ± 10.86 (46-83) |
| Sex, No. M/F     | 16/15             | 6/5               | 10/10             | 11/16             |
| Levodopa/DDI, mg | 473.21 ± 228.11 (150-900) | 330 ± 211.41 (150-800) | 552.78 ± 200.53 (200-900) | NA               |
| Duration of PD, y| 8.03 ± 6.10 (0.5-25.0) | 5.00 ± 5.06 (0.5-17.0) | 9.70 ± 6.09 (3-25) | NA               |
| UPDRS score      | 48.77 ± 28.51 (9-106) | 30.45 ± 24.09 (9-74) | 58.85 ± 22.42 (12-106) | NA               |
| HYS score        | 2.42 ± 1.02 (1-4) | 1.91 ± 0.97 (1-4) | 2.70 ± 0.95 (1-4) | NA               |
| Neuropathy signs, sum of scores† | 3.10 ± 2.04 (0-7) | 3.00 ± 1.61 (0-5) | 3.15 ± 2.28 (0-7) | NA               |
| Vibration perception† | 10.90 ± 2.27 (7-14) | 12.45 ± 1.44 (10-14) | 10.05 ± 2.16 (7-14) | NA               |
| tHcy, µmol/L     | 2.58 ± 0.93 (1.08-5.20) | 1.60 ± 0.31 (1.08-2.03) | 3.11 ± 0.69 (2.23-5.20) | 1.71 ± 0.46 (0.88-2.77) |
| SNAP, µV         | 14.97 ± 8.29 (4.1-41) | 21.52 ± 9.30 (7.3-41) | 11.36 ± 4.92 (4.1-21.1) | 28.07 ± 5.75 (19.5-40.5) |
| SCV, min/s       | 100.01 ± 9.72 (79.2-121.7) | 104.40 ± 9.37 (92.9-121.7) | 97.59 ± 9.26 (79.2-111.7) | 98.61 ± 6.04 (87.5-110.8) |

Abbreviations: DDI, dopa decarboxylase inhibitor; HYS, Hoehn and Yahr Scale; NA, not applicable; PD, Parkinson disease; SCV, sensory nerve conduction velocity; SNAPs, sensory nerve action potentials; tHcy, total homocysteine level; UPDRS, Unified Parkinson’s Disease Rating Scale.

SI units: To convert tHcy to micromoles per liter, multiply by 7.397.

*Unless otherwise indicated, data are expressed as mean ± SD (range). PD groups are described in the “Statistics” subsection of the “Methods” section.

†Scores are explained in the “Subjects” subsection of the “Methods” section.
in controls. The post hoc analysis showed significantly decreased SNAPs in PD patients with elevated tHcy levels compared with controls and PD patients with reference tHcy levels. Even the SNAPs of PD patients with reference tHcy levels were lower than those of controls (Table 2). The perception of vibration was significantly better in PD patients with reference tHcy levels than in those with elevated tHcy levels. No differences in SCV were found between PD patients and controls (Table 2).

No impact of covariates occurred in the whole analysis (results not shown).

In the correlation analysis, we found significant results only in the PD patients. There were significant associations between tHcy levels and SNAPs ($R = -0.52; P = .002$) and SCV ($R = -0.47; P = .008$). Daily levodopa/DDI intake was significantly related to tHcy levels ($R = 0.43; P = .02$), Unified Parkinson’s Disease Rating Scale total score ($R = 0.56; P = .002$), and duration of PD ($R = 0.49; P = .005$). Moreover, duration of PD and Unified Parkinson’s Disease Rating Scale total score significantly correlated with each other ($R = 0.49; P = .005$). The perception of vibration significantly correlated to the SNAP ($R = 0.55; P = .002$). We found no further significant relations (results not shown).

Our results indicate an indirect association between homocysteine metabolism and peripheral axonal accentuated neuronal degeneration, which corresponds to the decreased SNAPs and perception of vibration. To establish a cause-and-effect relationship, additional studies must show that vitamin supplements slow down or even reverse dysfunction of the sural nerve in PD.\(^{16,17}\) The significant increase of tHcy levels in PD patients and the significant correlations between tHcy levels, the electrophysiologic data, and daily levodopa dosage support our study outcomes. We assume that the individually varying gastrointestinal tract absorption of levodopa caused the missing relation between SNAP amplitudes and the daily levodopa dosage.\(^{18}\) Nevertheless, we have confirmed previous findings that long-term levodopa/DDI therapy is associated with elevation of tHcy levels.\(^{3,13}\)

Homocysteine induces marked cell injury and decreases nerve growth factor synthesis with its well-known trophic effects on sensory neurons and on the dopaminergic clonal line of rat adrenal pheochromocytoma cells that respond to nerve growth factor.\(^{8,19}\) We hypothesized that the observed homocysteine-associated peripheral SNAP decrease at least partially serves as a surrogate marker of steps of extracellularly located events, mostly centrally run via the apoptotic pathway in neurodegeneration, that cause the final intracellular step of neuronal cell death.\(^{3,8,11}\) Because of the overlap of SNAP amplitudes within the various groups, the measurement of SNAPs may be of limited clinical value. However, we believe that monitoring and subsequent therapeutic decrease of homocysteine levels are essential to prevent and/or delay peripheral axonal neuronal death, brain atrophy, and/or progression of PD.\(^{3,11}\) Putative therapeutic approaches for reduction of tHcy levels include vitamin supplementation because folic acid and cyanocobalamin catalyze and enhance metabolism of homocysteine to methionine.\(^{3,7,11,20,21}\) A further hypothetical therapeutic alternative would be application of peripherally acting COMT inhibitors as adjunctives to levodopa/DDI treatment. The COMT inhibitors increase the peripheral bioavailability of levodopa, but the combination of levodopa/DDI with COMT inhibitors reduces O-methylation of levodopa and thus hypothetically decreases homocysteine levels.\(^{3,4,7}\) In theory, well-titrated and carefully monitored SAM supplementation with normalization of reduced SAM levels in PD patients may also cause an effective reduction of homocysteine levels.\(^{4}\) However, elevated SAM dosages may also worsen motor symptoms or even accelerate neuronal cell death via the apoptotic pathway.\(^{5,22}\) Long-term studies are needed for all of these therapeutic approaches, which we hope will prevent neuronal dysfunction and subsequent degeneration.

**Comment**

The SNAP decrease is an electrophysiological sign of peripheral neuronal dysfunction. This circumstantial evidence suggests that, to a certain extent, SNAPs are a sur-

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**Table 2. Statistical Analysis of Sensory Nerve Conduction Findings, tHcy Levels, and Clinical Characteristics in PD Patients and Controls**

<table>
<thead>
<tr>
<th>ANCOVA</th>
<th>PD With Elevated tHcy Values vs Controls</th>
<th>PD With Elevated tHcy Values vs Reference tHcy Values</th>
<th>PD With Reference tHcy Values vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$ Value</td>
<td>$F$</td>
</tr>
<tr>
<td>Levodopa/DDI</td>
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<td>.03</td>
</tr>
<tr>
<td>UPDRS</td>
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<td>.004</td>
<td>.007</td>
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<tr>
<td>Neuropathy signs†</td>
<td>0.001</td>
<td>.93</td>
<td>.08</td>
</tr>
<tr>
<td>Vibration perception†</td>
<td>9.49</td>
<td>.005</td>
<td>.008</td>
</tr>
<tr>
<td>tHcy</td>
<td>46.12</td>
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<td>&lt;.001</td>
</tr>
<tr>
<td>SNAP</td>
<td>42.40</td>
<td>&lt;.001</td>
<td>.001</td>
</tr>
<tr>
<td>SCV</td>
<td>3.33</td>
<td>.57</td>
<td>.54</td>
</tr>
</tbody>
</table>

Abbreviations: ANCOVA, analysis of covariance; DDI, dopa decarboxylase inhibitor; NA, not applicable; NS, not significant; SCV, sensory nerve conduction velocity; SNAPs, sensory nerve action potentials; tHcy, total homocysteine level; UPDRS, Unified Parkinson’s Disease Rating Scale.

* Calculated by means of the Tukey Honestly Significant Difference test.
† Scores are explained in the “Subjects” subsection of the “Methods” section.

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**Conclusions**

Homocysteine induces marked cell injury and decreases nerve growth factor synthesis with its well-known trophic effects on sensory neurons and on the dopaminergic clonal line of rat adrenal pheochromocytoma cells that respond to nerve growth factor.\(^{8,19}\) We hypothesized that the observed homocysteine-associated peripheral SNAP decrease at least partially serves as a surrogate marker of steps of extracellularly located events, mostly centrally run via the apoptotic pathway in neurodegeneration, that cause the final intracellular step of neuronal cell death.\(^{3,8,11}\) Because of the overlap of SNAP amplitudes within the various groups, the measurement of SNAPs may be of limited clinical value. However, we believe that monitoring and subsequent therapeutic decrease of homocysteine levels are essential to prevent and/or delay peripheral axonal neuronal death, brain atrophy, and/or progression of PD.\(^{3,11}\) Putative therapeutic approaches for reduction of tHcy levels include vitamin supplementation because folic acid and cyanocobalamin catalyze and enhance metabolism of homocysteine to methionine.\(^{3,7,11,20,21}\) A further hypothetical therapeutic alternative would be application of peripherally acting COMT inhibitors as adjunctives to levodopa/DDI treatment. The COMT inhibitors increase the peripheral bioavailability of levodopa, but the combination of levodopa/DDI with COMT inhibitors reduces O-methylation of levodopa and thus hypothetically decreases homocysteine levels.\(^{3,4,7}\) In theory, well-titrated and carefully monitored SAM supplementation with normalization of reduced SAM levels in PD patients may also cause an effective reduction of homocysteine levels.\(^{4}\) However, elevated SAM dosages may also worsen motor symptoms or even accelerate neuronal cell death via the apoptotic pathway.\(^{5,22}\) Long-term studies are needed for all of these therapeutic approaches, which we hope will prevent neuronal dysfunction and subsequent degeneration.
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