Interference of Levodopa and Its Metabolites with Colorimetry of Uric Acid

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Reportedly, levodopa (L-DOPA) administration produces spuriously high values for plasma uric acid as measured by the commonly used phosphotungstic acid–hydroxylamine colorimetric method. We confirm this interference, not only by L-DOPA but also by three of its major metabolites: dopamine, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid. However, at therapeutic concentrations in plasma (<5 mg/L), the maximum spuruous uric acid concentration due to L-DOPA is <2 mg/L. Also, at reported peak plasma concentrations of L-DOPA plus three of its major metabolites, the maximum spuruous uric acid concentration due to all four compounds combined is <8.5 mg/L. Therefore, the hyperuricemia observed with this method in some patients who are chronically receiving L-DOPA cannot be attributed only to interference by L-DOPA and its metabolites in the colorimetric determination of uric acid. Evidently L-DOPA may increase laboratory values for plasma uric acid concentrations, both by pharmacological and chemical mechanisms.

Additional Keyphrases: variation, source of · hyperuricemia · dopamine · homovanillic acid · monitoring therapy

Several investigators have reported that chronic administration of levodopa (L-DOPA) to patients with Parkinson’s disease resulted in hyperuricemia and, occasionally, gout (1–3). This apparent hyperuricemia during L-DOPA administration has been attributed to interference by L-DOPA in the nonspecific colorimetric method used to determine concentrations of uric acid in plasma (4). Singh et al. (5) calculated a maximum extracellular fluid concentration of L-DOPA in humans, assuming that L-DOPA was distributed only in plasma and was not metabolized. If these assumptions are correct, oral ingestion of 1 g of L-DOPA could yield a plasma L-DOPA concentration as high as 83.3 mg/L. They demonstrated that this concentration of L-DOPA produced a spuruous uric acid concentration of 55 mg/L when uric acid was determined colorimetrically.

These results have been taken as sufficient to explain the reports of hyperuricemia in patients being treated with L-DOPA. However, reported plasma L-DOPA concentrations in patients who are being treated with L-DOPA are only 6% of those estimated by Singh et al. (6). In addition, L-DOPA is known to be rapidly metabolized in humans to dopamine, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid (6). Peak plasma concentrations of these metabolites range from 0.25 to 3.6 mg/L for dopamine (7, 8) and from 0.2 to 8.3 mg/L for 3,4-dihydroxyphenylacetic acid plus 3-methoxy-4-hydroxyphenylacetic acid (6). Because these metabolites are structurally similar to L-DOPA, they may also produce spuriously high uric acid values when uric acid is measured by this colorimetric method, but this has not previously been investigated. The present study was designed to investigate this and to compare these results with those obtained by the enzymic (uricase) procedure. The need to perform these studies arose after we found that L-DOPA and dopamine inhibit the renal tubular excretory transport of uric acid and that L-DOPA can produce hyperuricemia in the chicken (9, 10).

Materials and Methods

Uric Acid Analyses

Uric acid concentrations were determined both by the phosphotungstic acid/hydroxylamine colorimetric and the ultraviolet uricase (uric acid oxidase; EC 1.7.3.3) methods. The former method (11) is widely used for the clinical analysis of uric acid and has been automated (Technicon Instruments Corp., Tarrytown, NY 11059). In this method, sodium tungstate is reduced by uric acid to form the chromogen “tungsten blue.” The color of this reaction mixture is enhanced by adding hydroxylamine. In our experiments, we measured the absorbance of the chromogen at 690 nm with a Model 24 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA 92634).

In the ultraviolet uricase method, uric acid (maximal absorption at 292 nm) is reduced to allantoin (no absorption at 292 nm) in the presence of uricase. The decrease in the absorbance at 292 nm provides a direct measure of the amount of uric acid present in the buffered assay mixture (12, 13). Uric acid concentrations so determined were measured in a split-beam spectrophotometer (Model 576, Perkin-Elmer Corp., Oak Brook, IL 60521).

Preparation of Plasma Samples

Fresh human plasma, a gift of the American Red Cross, St. Paul, MN 55107, was stored at −20 °C until analysis, when it was thawed at ambient temperature and placed in an ice bath until use. Any precipitate in the thawed sample was allowed to settle before the supernatant liquid was sampled. To each 0.9-mL sample of human plasma, we added 0.1 mL of an aqueous solution of L-DOPA, dopamine, 3,4-dihydroxyphenylacetic acid, or 3-methoxy-4-hydroxyphenylacetic acid. Samples were mixed and analyzed immediately by the phosphotungstic acid/hydroxylamine and (or) ultraviolet uricase methods.

Plasma samples from parkinsonian patients who were receiving between 750 and 1500 mg of L-DOPA daily in combination with 75 to 150 mg of carbidopa (Merck, Sharp and Dohme, West Point, PA 19568) were supplied by Dr. D. B. Calne (National Institutes of Health, Bethesda, MD). Plasma was sampled 1 to 3 h after the morning L-DOPA administration. Samples were received frozen and analyzed for uric acid by both the above methods immediately after thawing.

Materials

Levodopa was generously supplied by Hoffman-La Roche, Nutley, NJ 07110. Uric acid, uricase, phosphotungstic acid solution, and dopamine were purchased from Sigma Chemical Co., St. Louis, MO 63178; 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid from Calbiochem-Behring Corp., San Diego, CA 92112.

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Results

Interference of L-DOPA with the Analysis for Uric Acid

L-DOPA was added to three samples of human plasma that contained no L-DOPA, to give concentrations ranging from 0 to 100 mg/L. The results obtained when these were analyzed by the uricase method were the same as those obtained for plasma samples that contained no L-DOPA (Figure 1). In contrast, results for the same plasma samples by the colorimetric method were considerably greater in those plasma samples that contained L-DOPA than in those that did not. This difference between the apparent and the actual uric acid concentration ("spurious" uric acid) is in agreement with Cawein and Hewins (4).

Singh et al. (5) calculated that, in humans, the extracellular fluid concentration of L-DOPA after oral administration of 250 or 1000 mg of L-DOPA would be 20.8 or 83.3 mg/L, respectively. When they analyzed aqueous solutions at these concentrations by use of the colorimetric method, they observed spurious uric acid concentrations of 7 and 35 mg/L, respectively; we (Figure 1) found corresponding spurious uric acid concentrations of 8 and 32 mg/L. However, the actual peak plasma L-DOPA concentrations reported in patients chronically receiving L-DOPA orally range from 0.2 to 5.0 mg/L (8), at most only 6% of the plasma L-DOPA concentration assumed by Singh et al. As shown in Figure 1, within the therapeutic plasma concentration range for L-DOPA, the maximum spurious uric acid due to L-DOPA is <2 mg/L. This small increase in the apparent plasma uric acid concentration is not sufficient to explain the hyperuricemia reported in patients receiving L-DOPA.

Interference of Metabolites of L-DOPA with the Analysis for Uric Acid

Figure 2 shows the effects of L-DOPA, dopamine, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid in the phosphotungstic acid/hydroxylamine colorimetric determination of uric acid. The latter three compounds were separately added to three human plasma samples so that the concentration of each ranged from 0 to 10 mg/L of plasma. Each produced a significant positive bias in the colorimetric determination of uric acid.

The maximum interference of these metabolites in the colorimetric assay can be calculated from their reported peak therapeutic plasma concentrations. Parkinsonian patients on long-term L-DOPA therapy have peak plasma dopamine concentrations of <4 mg/L (7, 8). Figure 2 shows that a plasma dopamine concentration of 4 mg/L would produce a spurious uric acid concentration of approximately 2.5 mg/L. Plasma 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid concentrations are considered to be reciprocally related because parkinsonian patients with the highest plasma concentrations of 3,4-dihydroxyphenylacetic acid have the lowest concentrations of 3-methoxy-4-hydroxyphenylacetic acid, and vice versa. The combined concentrations of 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid in plasma reportedly do not exceed 8.3 mg/L (6), which would produce a maximum spurious uric acid concentration of between 3 and 4 mg/L. Therefore, when the spurious uric acid concentrations produced by dopamine (2.5 mg/L) and 3,4-dihydroxyphenylacetic acid plus 3-methoxy-4-hydroxyphenylacetic acid (3 to 4 mg/L) are added to that produced by L-DOPA (2 mg/L), the maximum interference is <8.5 mg/L. Even this would require the simultaneous presence of maximum plasma concentrations of L-DOPA, dopamine, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid.

We analyzed the uric acid concentration of plasma from four parkinsonian patients receiving L-DOPA to determine the magnitude of the spurious uric acid (Table 1). Each of these patients received between 750 and 1500 mg of L-DOPA daily in combination with 75 to 150 mg of carbidopa. The second patient listed in Table 1 also received 100 mg of bromocriptine in addition to L-DOPA and carbidopa. We investigated the possible interaction of carbidopa and bromocriptine in the analysis of uric acid. At a maximum therapeutic concentration of carbidopa in plasma, 2 to 3 mg/L (14), and a bromocriptine concentration of 1000 mg/L, neither of these compounds interfered notably with the colorimetric analysis for uric acid. When we compared the results obtained by the nonspecific phosphotungstic state method and the more specific uricase method, we found no significant difference (Table 1). This agrees with the findings of Fermaglich et al. (15). These

Table 1. Plasma Uric Acid Concentration of Parkinsonian Patients: Results by the Colorimetric and Ultraviolet Uricase Methods Compared

<table>
<thead>
<tr>
<th>Plasma uric acid concn., mg/L (and SD)</th>
<th>Uricase</th>
<th>Colorimetric</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td>53.8 (0.6)</td>
<td>53.1 (2.4)</td>
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<tr>
<td>57.7 (1.5)</td>
<td>57.6 (0.6)</td>
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<tr>
<td>78.0 (2.0)</td>
<td>81.1 (0.6)</td>
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<tr>
<td>34.3 (0.8)</td>
<td>31.3 (0.3)</td>
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data suggest that the actual spurious uric acid due to therapeutic amounts of L-DOPA and its metabolites in the colorimetric determination of uric acid is much less than 8.5 mg/L.

Discussion

Our results confirm the reported interference of L-DOPA with the colorimetric determination of plasma uric acid (4, 5), but in addition we have shown that three major metabolites of L-DOPA also interfere. However, our results do not agree with the conclusions of Cawein and Hewins (4) and Singh et al. (5) that increases in apparent uric acid concentrations in the plasma of patients receiving L-DOPA can be explained by the interference of L-DOPA in the colorimetric determination of uric acid. Their conclusion was not based on the interference of therapeutic L-DOPA and metabolite concentrations in plasma but rather on unquantitated observations (4) and on an overestimate of therapeutic plasma L-DOPA concentrations (5). Peak plasma L-DOPA concentrations of patients who are chronically receiving L-DOPA are only 6% as great as assumed by Singh et al. (8). However, increases in plasma uric acid concentrations exceeding 8.5 mg/L have been reported in patients receiving L-DOPA (7).

We previously reported that L-DOPA and dopamine decreased the renal tubular excretory transport of [14C]uric acid and that L-DOPA produced hyperuricemia in the chicken (9, 10). Renal tubular excretory transport of uric acid in the chicken has many similarities to that of humans (16–18). Thus, in addition to producing a positive bias in the colorimetric analysis of uric acid, L-DOPA and its metabolites may also inhibit the renal tubular excretory transport of uric acid and thereby produce a hyperuricemia.

Our data indicate that interference of L-DOPA in the colorimetric determination of uric acid cannot totally account for the reported hyperuricemia in patients who are chronically receiving L-DOPA. Many physicians may wish to treat asymptomatic hyperuricemia. However, because of the mistaken belief that patients receiving L-DOPA will show a false hyperuricemia, patients who would normally be treated for hyperuricemia may remain untreated, especially elderly men, in whom the risk of hyperuricemia is greatest (19) and the incidence of parkinsonism high.

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References


