Plasma L-Dopa in the Diagnosis of Malignant Melanoma

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We determined the concentration of L-dopa in the plasma of 98 patients with biopsy-proven melanoma, a dermatological neoplasm that is characterized biochemically by abnormal tyrosine metabolism. For 21 patients previously diagnosed as having melanoma but who were clinically free of disease (stage I), the mean concentration of L-dopa in plasma, 1.01 (SD 0.12) μg/L, was not significantly different from that of 32 normal controls, 1.23 (SD 0.16) μg/L. However, L-dopa was increased significantly (p < 0.001) in the plasma of all of 65 patients with active disease (stage II), 2.08 (SD 0.46) μg/L, and was highest in 12 patients with stage III malignant melanoma, 8.40 (SD 3.50) μg/L. The development of metastases in four patients with stage II melanoma was accompanied by an increase in the concentration of plasma L-dopa. These studies suggest that measurement of plasma L-dopa may be useful in the diagnosis of melanoma.

Additional Keyphrases: cancer · plasma vs urine samples · radioenzymatic assay · melanogens · tumor marker

Melanomas are malignant tumors arising from melanin-producing cells, melanocytes. The tumor is characterized biochemically by abnormal tyrosine metabolism (I, 2). The urine of some patients with far-advanced melanoma is dark, or darkens on standing. This melanuria—or, more properly, melanogenuria—occurs in only a small proportion of melanoma patients, usually at a very far advanced stage, who survive long enough to develop the requisite tumor burden. In addition, most of these patients excrete large amounts of phenol and indole melanogens in their urine. Voorhees (3) and Turer and Kasel (4) found an increased output of L-dopa in 24-h urine specimens from patients with malignant melanoma, and they demonstrated that the values returned towards normal after treatment. Consequently, the capability of the melanoma tumor to secrete increased quantities of the melanin precursor, L-dopa, and its metabolites in urine may be regarded as a specific biochemical marker for diagnosis. However, certain problems with measuring urinary L-dopa limit its application to the diagnosis of melanoma in many cases. These may include incomplete collection of 24-h urine specimens, cumbersome collection procedures, and tedious sample preparation for routine analysis, the normal concentration of L-dopa (0.5–1.5 μg/24 h) being too low for direct assay in diluted urine specimens. Furthermore, kidney dysfunction in patients with melanoma interferes with the urinary excretion of L-dopa.

Our main objective here was to determine whether direct measurement of L-dopa in plasma by a sensitive and specific radioenzymatic technique (5) would reliably indicate malignant melanoma. Accordingly, we have determined the concentration of L-dopa in samples of plasma from 98 patients with biopsy-proven melanoma. We also evaluated the concentrations of L-dopa in a subgroup of stage II patients who later developed metastases.

Materials and Methods

Patients

The melanoma patients we studied had been admitted to the Oncology Service of Emory University Hospital for surgical treatment. We collected venous plasma from four groups of subjects, ages 20 to 70 years.

Group A consisted of 21 patients admitted to the hospital for a wide excision of a previously biopsied primary melanoma; in all cases, pathological evaluation of the excised tissue revealed malignant melanoma. Group B consisted of 65 patients with stage II malignant melanoma, the stage at which the melanoma presents as a palpable skin lesion with metastases to regional lymph nodes, requiring a therapeutic radical node dissection. Group C consisted of 12 patients with clinical stage III disease. In stage III disease, the melanoma has metastasized to distant organs and (or) to multiple lymph node areas, or it has spread diffusely in the skin and subcutaneous tissue of the involved limb or region of the body. The clinical diagnosis of all three stages was confirmed by microscopic examination of the involved nodes. The presence and location of metastases was established by chest roentgenography, bone scan (99mTc-methylene diphosphate), liver scan (99mTc-sulfur colloid), and computed tomography of the brain. The control group consisted of 32 apparently healthy adults.

We also conducted a follow-up study of L-dopa in plasma in four patients from Group B who subsequently developed distant metastases and had shifted five to 15 months later from stage II malignant disease into stage III.

Analysis

We collected 3 mL of blood into evacuated glass tubes containing glutathione and (ethylenebis(oxymetheninitrilo)tetraacetic acid (EGTA; Upjohn Co., Kalamazoo, MI), chilled the samples to 0 °C, and centrifuged immediately at 500 × g for 10 min. The plasma was then removed and quickly frozen at −20 °C until analysis within two weeks. We determined the concentration of L-dopa in plasma by the radioenzymatic procedure of Johnson et al. (5). In brief, their procedure is as follows: 50 μL of plasma is incubated (37 °C, 40 min) in the presence of dopa decarboxylase (EC 4.1.1.28), which is used to convert L-dopa in the sample to dopamine, which in turn is converted to 3-O-methyl-3H-methyl-dopa in the presence of catechol-O-methyltransferase (EC 2.1.1.6) and S-[methyl-3H]adenosylmethionine. The methylated metabolite formed is extracted and characterized by radiochromatographic analysis as described by Feuler and Johnson (6). As little as 10 pg/50 μL, for L-dopa per sample, can be detected. Intra- and interassay CVs were 10 and 12%, respectively, for L-dopa concentrations of 1.0 to 2.5 μg/L.

Statistical Analysis

We compared the stage of the disease and the concentration of L-dopa in plasma of melanoma patients by using the Kruskal-Wallis test, followed by multiple pairwise comparison tests. Results of the follow-up study on four subjects were evaluated with Student's paired t-test. Values of p < 0.05 were considered statistically significant.

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31x5 to 581x787

Results

In the first part of this study, we determined the concentration of L-dopa in venous plasma from normal subjects and melanoma patients. In patients who had been previously diagnosed as having melanoma, but were currently free of disease (stage I), the mean (± SD) L-dopa concentration (1.01 ± 0.12, µg/L) was not significantly different from that of normal controls (1.23 ± 0.21, µg/L). However, it was significantly (p < 0.001) increased in patients with stage II malignant melanoma (2.08 ± 0.46 µg/L) (Figure 1). The L-dopa concentration was highest in the plasma of the 12 patients with stage III malignant melanoma (8.40 ± 3.55 µg/L).

In the follow-up study, we found that the development of metastases in patients with documented stage II melanoma was associated with highly significant (p < 0.002) increases in L-dopa concentrations (Table 1). L-Dopa in plasma increased from 1.62 (SD 0.263) µg/L during stage II to 6.97 (SD 0.78) µg/L during stage III.

Discussion

The increasing incidence of malignant melanoma and its associated mortality (7–9) accentuate the need for a specific, clinically reliable biochemical "marker," capable of predicting its severity and early detection of metastases. Because the biochemistry of malignant melanoma is essentially characterized by accelerated production of L-dopa, a key intermediate in melanin biosynthesis, we measured L-dopa in the plasma of melanoma patients, finding significant increases in all melanoma patients with active disease at the time of diagnosis. In contrast, its concentration was normal in plasma of disease-free melanoma patients with previously excised lesions. Patients with more disseminated disease had the highest concentrations of L-dopa. The utility of plasma L-dopa measurement in the management of this tumor became evident when four patients with stage II melanoma exhibited a four- to sixfold increase of L-dopa in plasma after the development of widespread metastases. However, serial samples were not obtained, so we cannot assess whether the increase in plasma L-dopa preceded or even paralleled the appearance of clinically recognizable metastases. If it did, this would be of great value in the long-term management of such patients. Further prospective studies are needed to address this issue.

What are the biochemical mechanisms of the increase of L-dopa in plasma in malignant melanoma? The pathway involved in melanin formation is relatively well understood (7). Although details of melanin synthesis are yet to be worked out, the rate-limiting step in melanogenesis seem to be the oxidation of tyrosine to L-dopa and of L-dopa to dopaquinone within the melanocytes—both reactions catalyzed by tyrosinase (monophenol monooxygenase, EC 1.14.18.1). The amount of tyrosinase activity in the cell is regulated by melatonin (melanocyte-stimulating hormone) (8). Various investigators have attempted to correlate serum tyrosinase assay activity with melanoma. Nishioka et al. (9) found significant increases of this enzyme in

Table 1. Follow-up Study of Four Patients with Melanoma

<table>
<thead>
<tr>
<th>Sex/age</th>
<th>Date of assay</th>
<th>Location of primary lesion</th>
<th>Clinical condition</th>
<th>Treatment</th>
<th>L-Dopa, µg/L</th>
<th>µg/L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/61</td>
<td>12/15/81</td>
<td>Thigh</td>
<td>Primary lesion and simultaneous involvement of left inguinal and retroperitoneal nodes Brain metastases</td>
<td>Wide local excision</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>05/14/82</td>
<td></td>
<td></td>
<td></td>
<td>6.60</td>
<td>6.60</td>
</tr>
<tr>
<td>3/43</td>
<td>10/01/82</td>
<td>Nodular</td>
<td>Multiple lymph node involvement Brain, liver, skin, and adrenal metastases</td>
<td>Regional lymphadenectomy</td>
<td>1.50</td>
<td>8.05</td>
</tr>
<tr>
<td></td>
<td>03/18/83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/32</td>
<td>05/20/82</td>
<td>Right suprascapular area</td>
<td>Disease free, no evidence of node involvement Brain, liver and pelvic metastases</td>
<td>Wide local excision</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>08/04/83</td>
<td></td>
<td></td>
<td></td>
<td>6.28</td>
<td>6.28</td>
</tr>
<tr>
<td>3/60</td>
<td>09/11/81</td>
<td>Left forearm</td>
<td>Seven years after resection of primary lesion, recurrent disease in left axilla and left cervical node</td>
<td>Left axillary lymph node dissection</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>07/23/82</td>
<td></td>
<td></td>
<td></td>
<td>7.00</td>
<td>7.00</td>
</tr>
</tbody>
</table>

*Single determinations.
patients with malignant melanoma. Moreover, increased tyrosinase activity was specific to melanoma patients, because it could not be detected in human cell lines from carcinoma of the breast and colon (9). Therefore, increased tyrosinase activity could accelerate the conversion of tyrosine to L-dopa, which would then accumulate in the blood or be excreted in abnormal amounts in the urine of patients with malignant melanoma.

Our findings of a significant increase in plasma L-dopa in melanoma patients with active disease agree with previous reports regarding the role of L-dopa in melanoma. Faraj et al. (10), Trapenznikov et al. (11), and Morgan et al. (12) demonstrated that in patients with metastases to regional lymph nodes, as well as those with locally disseminated forms of malignant melanoma, the urinary excretion of L-dopa and its metabolites is increased significantly.

In summary, radioenzymatic measurement of L-dopa in plasma may have diagnostic implications in melanoma in that it may foretell the development of metastases in patients with primary or regionally confined stage I or II disease (13–15).

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References