Dietary Influence of Tyrosine and Phenylalanine on the Response of B16 Melanoma to Carbidopa-Levodopa Methyl Ester Chemotherapy

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ABSTRACT

The effect of dietary tyrosine and phenylalanine on survival of adult female C57BL/6 × DBA/2 F1, mice bearing i.p. slow-growing, moderately pigmented and fast-growing, highly pigmented B16 melanoma tumors was studied alone and in combination with carbidopa-levodopa methyl ester chemotherapy. These studies tested three different diets: a natural product diet containing 1.09% phenylalanine and 0.64% tyrosine (commercial diet); a chemically defined crystalline amino acid diet containing 0.6% phenylalanine and 0.3% tyrosine (purified diet); and a nutritionally deficient chemically defined diet containing 0.08% phenylalanine and 0.04% tyrosine (deficient diet). Mice received carbidopa (100 mg/kg) and levodopa methyl ester (1000 mg/kg) i.p. daily for 12 days.

The median survival of mice bearing the slow-growing tumor averaged 8 days longer than that of mice bearing the fast-growing tumor. Median survival increased by 42% (slow-growing tumor) and by 30% (fast-growing tumor) in mice maintained in the purified diet. Drug treatment significantly increased survival in mice maintained on the purified and deficient diets. Drug treatment was relatively ineffective in mice maintained on the commercial diet regardless of the tumor growth characteristics. The largest increases in median survival of 61% (slow-growing tumor) and of 78% (fast-growing tumor) occurred in the treated mice maintained on the deficient diet. Plasma tyrosine and phenylalanine levels decreased by 33 and 21%, respectively, in mice maintained on the deficient diet and were unaffected by drug treatment. Tumors were 60% smaller in mice maintained on the deficient diet compared to mice maintained on the purified diet.

Drug treatment resulted in decreased food intake and weight loss in all tumor-bearing dietary groups. Restricting food intake in untreated tumor-bearing mice to the amounts consumed by the drug treatment groups resulted in a parallel loss in body weight but no significant alteration in median survival. These data show that concomitant dietary tyrosine-phenylalanine restriction enhances the antitumor activity of carbidopa-levodopa methyl ester against B16 melanoma.

INTRODUCTION

Both levodopa treatment and tyrosine and/or phenylalanine restriction have inhibitory effects on the growth of malignant melanoma. Levodopa and related catechol compounds show significant antitumor activity in a variety of experimental animal tumor systems (7, 20, 41–44, 48, 49) and recently in the treatment of human melanoma patients (45, 46). One analog, levodopa methyl ester, both alone and in combination with the dopa decarboxylase inhibitor, benserazide, is effective in the pigmented B16 melanoma system (41). In vitro observations suggest that this antitumor activity results from the in situ generation of reactive quinone intermediates that preferentially inhibit DNA synthesis (47).

The in vivo growth of B16 melanoma is inhibited by depletion of tyrosine and/or phenylalanine. Restriction of these 2 amino acids, either through dietary deficiency (5, 6, 8, 16, 18) or by administration of specific degrading enzymes (10, 11, 21), inhibits growth of both experimental and human melanomas. Tyrosine depletion in vitro has a cytostatic effect on B16 melanoma cells and is a strong inhibitor of RNA synthesis (9).

Dietary restriction of tyrosine and phenylalanine also alters host immune responses (3, 17, 23). The exact mechanism whereby dietary restriction of tyrosine and phenylalanine creates this inhibitory environment for tumor growth remains uncertain.

Dietary tyrosine and phenylalanine and perhaps other large neutral amino acids may actually modulate the antitumor activity of levodopa. Consuming high-protein diets consisting of high levels of tyrosine and phenylalanine impairs both the adsorption and therapeutic effect of levodopa in human Parkinsonism patients (4, 15), whereas low-protein diets tend to potentiate and stabilize the therapeutic effects of this drug (4, 15, 22). Although the mechanism responsible for the impaired response in patients consuming the high-protein diets is not definitively known, it is well established that the aromatic amino acids tyrosine, phenylalanine, levodopa, and tryptophan and the branched-chain amino acids leucine, isoleucine, and valine compete for absorption at both the blood-brain barrier and at neuronal membranes. The apparent competition results because these amino acids share a common uptake mechanism (13, 24, 50). The antitumor activity of levodopa is dependent upon selective incorporation into melanoma cells (41, 42, 49), and amino acid competition may interfere with the effectiveness of this drug.

The importance of large neutral amino acids to the antitumor effect of various other drugs is also known. The uptake and cytotoxicity of L-phenylalanine mustard against murine L1210 leukemia cells are inhibited competitively by leucine (39). The antitumor activity of azaserine is more effective in animals maintained on an isoleucine-deficient diet (33). The amino acid analog, p-fluorophenylalanine, is totally dependent on concurrent dietary restriction of phenylalanine for antitumor activity against BW7756 hepatoma and C3HBA mammary adenocarcinoma tumors (30). Phenylalanine imbalance as an adjunct to non-amino acid analog therapy greatly enhances the response of human patients receiving combination chemotherapy with mitomycin C, 5-fluorouracil, 1-β-D-arabinofuranosylcytosine,
and tolyomyacin in individuals receiving single-drug therapy with 6-mercaptopurine or steroids (19).

In this study, we examine the combined antitumor activity of levodopa methyl ester administered with the dopa decarboxylase inhibitor, carbidopa, combined with dietary restriction of tyrosine and phenylalanine. This approach offers the advantage of utilizing different antitumor mechanisms concomitantly and avoids the potential competition for uptake into melanoma cells between dietary amino acids and the drugs. Since carbidopa is a competitive inhibitor of tyrosine aminotransferase (37) and since administration of this drug can result in a 2- to 3-fold increase in endogenous serum and tissue levels of tyrosine (1, 51), limitation of dietary tyrosine and phenylalanine should enhance the effect of the antitumor drug.

MATERIALS AND METHODS

Chemicals. All chemicals and drugs used in this study were reagent grade. Levodopa methyl ester was purchased from Sigma Chemical Company, St. Louis, Mo. Carbidopa was a gift from Merck Sharp and Dohme, West Point, Pa.

Mice. Specific-pathogen-free female C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) mice were purchased from Harlan/Sprague-Dawley, Madison, Wis., at 4 to 6 weeks of age. Adult female mice at 14 to 16 weeks of age and averaging 21.1 ± 1.2 (S.D.) g were used for all experiments. The mice were maintained ad libitum on a locally manufactured natural product diet containing 18.7% crude protein, 2.6% crude fat, and 6.7% crude fiber until distributed into experimental dietary groups. Mice were accustomed to the test diets for at least 2 weeks before antitumor experiments.

The animal facility is accredited by the American Association for Accreditation of Laboratory Animal Care and conforms to their standards for the care and use of laboratory animals. The mice were housed in separate quarters where temperature was controlled to ±1° and humidity was controlled to ±5%. There were 6 to 10 air changes/hr, and the mice were kept on a 12-hr-light, 12-hr-dark cycle with light from 6 a.m. to 6 p.m. Experimental mice were pair-housed in suspended stainless steel cages (20 x 25 x 18 cm) with mesh floors and sides unless otherwise indicated. Each cage was also provided with Nestlet bedding (Ancare Corporation, Manhasset, N. Y.). Food intake and water were measured in glass cylindrical drinking tubes calibrated to ±1 ml (BioServ, Inc., Frenchtown, N. J.).

Test Diets. LabBlox Sterilizable Animal Diet commercial diet was obtained from Allied Mills, Chicago, Ill. This standard natural-product laboratory chow consists of 24% crude protein, 4% crude fat, and 4.6% crude fiber and provides 3.86 kcal/g of food. This diet contains 1.09% L-phenylalanine and 0.64% tyrosine, which is of particular significance to this study.

The synthetic diet (purified diet) was composed of crystalline amino acids and was prepared by BioServ, Inc. It is adequate for growth, maintenance, and reproduction of B6D2F1 mice. This diet is equivalent in protein to an 11.8% casein diet, provides approximately 4.0 kcal/g, and is similar in amino acid composition to the diets described by Bouhours and Kongsvang (3) and by Theuer (36). The diet contained 0.6% L-phenylalanine, 0.3% L-tyrosine, 0.5% L-isoleucine, 0.8% L-leucine, 0.9% L-lysine, 0.4% L-methionine, 0.5% L-threonine, 0.7% L-valine, 0.15% L-tryptophan, 0.3% L-histidine, 0.21% L-arginine, 0.2% L-cystine, 0.53% L-alanine, 1.23% L-aspartic acid, 0.23% L-glutamic acid, 1.96% L-proline, 1.04% L-serine, and 4.8% L-glutamic acid. The diet also contained 10% corn oil as a source of fat, 6.0% cornstarch, 15% dextrose, and 40.96% sucrose as sources of carbohydrates, and 5% cellulose as a source of fiber. Five % HMW Salts Modified to Meet NRC Rat and Mouse Requirements was added as a source of minerals. The salt mixed consisted of (g/kg): dibasic calcium phosphate, 45.8; calcium carbonate, 207.917; sodium chloride, 112; monobasic potassium phosphate, 154; magnesium carbonate, 25.2; magnesium sulfate, 16; ferric phosphate, 20.8; potassium iodide, 0.08; manganese sulfate, 4.7; sodium fluoride, 0.12; aluminum potassium sulfate, 0.16; cupric sulfate, 0.86; zinc carbonate, 2.14; and sodium selenite, 0.003. A 2.2% Diet Fortification Bio-Mix No. 20315 was added as a source of vitamins and consisted of (g/kg): vitamin A, 4.5 (200,000 IU/g); vitamin D, 0.25 (400,000 IU/g); α-tocopherol, 5.00; ascorbic acid, 45.00; l-tryptophan, 5; choline chloride, 75; menadione, 2.25; p-aminobenzoic acid, 5; niacin, 4.5; riboflavin, 1.00; pyridoxine-HCl, 1.00; thiamine-HCl, 1.00; calcium pantothenate, 3.00; biotin, 0.020; folic acid, 0.009; and vitamin B12, 0.00135. The diet also contained 0.015% α-tocopherol acetate and 2% sodium bicarbonate for preservation of labile constituents and pH control.

The deficient diet was prepared by reducing the tyrosine and phenylalanine content of the purified diet to 0.08% phenylalanine and 0.04% tyrosine. Glycine and glutamic acid were altered to make this diet isonitrogenous to the purified diet. Both diets were stored in the dark in tightly sealed containers at 4° to minimize deterioration (35) and used within 90 days after purchase. All diets were administered in pelleted form, and fresh pellets were offered daily. Food was removed from mice at least 8 hr prior to collection of blood. Blood was collected at the same time (9 a.m.) of day to avoid changes in plasma amino acid levels due to diurnal variation (12). Mice were mated by the orbital technique (25). Blood from groups of 4 to 6 mice was collected in heparinized capillary tubes, transferred to chilled microcentrifuge tubes, and centrifuged. A known weight of plasma was processed for amino acids using the perchloric acid method of Saifer (31). The processed samples were lyophilized at −50° in a VirTis lyophilizer and stored at −70° prior to analysis on a Beckman Model 121 MB amino acid analyzer. Norleucine was added as an internal standard.

Tumor. B16 melanoma tumors from 2 different sources were used in these studies. Results with a moderately pigmented, slow-growing B16 melanoma tumor obtained through the courtesy of Dr. Vernon Riley, Fred Hutchinson Cancer Research Center, Seattle, Wash., are presented in Tables 1 and 2 and in Chart 1. Results with a faster-growing, highly pigmented tumor obtained from the Mason Research Institute, Worcester, Mass., are presented in Table 3 and Chart 2. This tumor was used for determining the effect of food restriction on survival of tumorbearing mice. Both tumors were maintained in vivo as s.c. transplants. All injected tumor suspensions, prepared according to the method of Fidler et al. (14), were greater than 98% viable by trypan blue exclusion tests. Mice received 106 viable cells i.p. in a fixed volume of 0.1 ml. Assay of tumor-bearing mice for lactate dehydrogenase (2) suggested the presence of the ubiquitous lactate dehydrogenase-elevating virus, but its actual presence was not confirmed. Although this virus is known to be present in many transplantable tumors (29), it does not significantly affect the growth rate of the pigmented B16 melanoma (28) but can cause immunological and other alterations in the host (26, 27).

Antimelanoma Experiments. Mice receiving the commercial diet were stabilized on the diet for 3 weeks before tumor inoculation. All other mice were initially stabilized for 1 week on the purified diet. One-half of the animals were continued on this diet, and one-half were placed on the deficient diet for an additional 2-week period. Mice were allowed free access to all diets throughout the experiments except for those mice involved in the diet restriction study. Water was freely available to mice at all times. Test mice were given injections of B16 melanoma after the stabilization period on Day 0. Treated groups received daily carbidopa-levodopa methyl ester therapy beginning 24 hr following tumor inoculation and continuing for 12 days. Carbidopa (100 mg/kg) was administered 90 min prior to injection of levodopa.
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Table 1
Effect of diet and drug treatment on survival and tumor weight in mice bearing the slow-growth B16 melanoma tumor

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group</th>
<th>Mean survival (days)</th>
<th>Median survival (days)</th>
<th>Increase in survival (%)</th>
<th>Mean tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>Untreated (22)</td>
<td>37 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36</td>
<td>16</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Treated (12)</td>
<td>40 ± 1</td>
<td>41</td>
<td>32</td>
<td>6.7 ± 0.9</td>
</tr>
<tr>
<td>Purified</td>
<td>Untreated (26)</td>
<td>32 ± 1</td>
<td>31</td>
<td>26</td>
<td>8.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Treated (16)</td>
<td>40 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39</td>
<td>26</td>
<td>7.9 ± 0.8</td>
</tr>
<tr>
<td>Deficient</td>
<td>Untreated (22)</td>
<td>46 ± 2</td>
<td>44</td>
<td>42</td>
<td>3.3 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Treated (16)</td>
<td>53 ± 2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50</td>
<td>61</td>
<td>3.4 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Untreated groups are composed of 0.9% NaCl solution-injected and uninjected mice. The groups are combined since statistical analysis indicated no significant difference in the mean survival. Treated groups were given injections of drug from Days 1 to 12 post-tumor inoculation as outlined in "Materials and Methods."

<sup>b</sup> % of increase = \( \frac{\text{Median survival of dietary untreated or treated group} - 1}{\text{Median survival of purified diet-untreated group}} \) × 100

<sup>c</sup> Tumor weight determined at necropsy in animals that died from tumor, mean ± S.E.

<sup>d</sup> Numbers in parentheses, number of mice in each group.

<sup>e</sup> Mean ± S.E.

<sup>f</sup> Values significantly different from untreated mice within the same dietary group (p < 0.01).

<sup>g</sup> Significantly different from all commercial and purified dietary groups (p < 0.01).

Table 2
Food consumption in drug treated non-tumor-bearing and tumor-bearing mice inoculated with the slow-growing tumor

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Treatment period</th>
<th>Posttreatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Tumor&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Commercial</td>
<td>3.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Purified</td>
<td>2.7 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Deficient</td>
<td>2.7 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from initial food consumption within dietary group (p < 0.001).

<sup>b</sup> Mean ± S.E.

<sup>c</sup> Significantly different from commercial diet group (p < 0.001).

methyl ester (1000 mg/kg). Both agents were prepared daily in 0.9% NaCl solution and injected i.p. in a volume equal to 1% of mouse body weight. Untreated groups were given injections of 0.9% NaCl solution. In order to assure quality control, some mice were not given injections. Some dietary and treated groups consisted of both tumor-bearing and non-tumor-bearing mice. The antitumor response was determined from the mean and median survival time.

**Diet Restriction Study.** All mice were single housed in suspended stainless steel cages and maintained on the purified diet until their weight stabilized. One-half were continued on this diet, and one-half were placed on the deficient diet for an additional 2 weeks. Untreated, treated, and restricted subgroups each containing 10 mice were formed within the dietary groups and inoculated with the fast-growing tumor on Day 0. The untreated and treated subgroups were allowed free access to the diets and were handled as outlined under "Antimelanoma Experiments." Beginning on Day 0, food intake was limited to 1.6 ± 0.1 (S.E.) g for 12 days and to 2.5 ± 0.1 g thereafter in the restricted subgroup receiving the purified diet; intake was limited to 2.0 ± 0.1 g throughout the experiment in the restricted subgroup receiving the deficient diet. These levels were selected to parallel the intake of the drug-treated mice bearing the slow-growing tumor (Table 2). Both restricted subgroups were given injections of 0.9% NaCl solution from Days 1 to 12.

**Statistical Analysis.** Significance between 2 means was determined by using Student’s t test. All multiple pairwise comparisons between more than 2 means were made using the least-significant-difference test after analysis of variance (34).

**RESULTS**

Slow-growing Moderately Pigmented Tumor. The data presented in Table 1 show the effect of diet and drug treatment on survival of mice implanted with the slow-growing tumor. Three groups were maintained on commercial laboratory chow, the purified diet, and the deficient diet. The similarity between the mean and median survival times indicates that the tumor grew uniformly in both untreated and treated dietary groups. Diet alone altered survival. Untreated mice maintained on the commercial diet showed a slight (16%) increase in median survival compared to those maintained on the purified diet. A 42% increase occurred in control animals fed the deficient diet alone. Drug treatment increased median survival in all dietary groups, with the deficient dietary group exhibiting the greatest increase (61%). Statistical analysis of the mean survival within dietary groups indicated no therapeutic effect in mice fed the commercial diet but significant therapeutic effects in both the purified and deficient dietary groups.

The weight changes observed in treated and untreated tu-
The most profound changes in weight resulted from the drug treatment, and both tumor-bearing and non-tumor-bearing mice lost weight in all dietary groups. Tumor-bearing mice maintained on the purified diet lost the most weight. Mice fed the purified and commercial diets regained the weight after drug treatment was stopped. Non-tumor-bearing mice retained weight at a faster rate than did tumor-bearing mice. Mice fed the deficient diet were unable to regain fully the weight lost during treatment regardless of tumor presence.

Mice maintained on the commercial diet consumed significantly more diet than did mice maintained on the purified or deficient diets before tumor inoculation (Table 2). The purified and deficient groups, however, readily accepted the diets. The tumor did not affect food consumption in non-drug-treated mice until the last 1 to 3 days before death when some mice became anorectic. Drug treatment significantly reduced food consumption in tumor-bearing mice by 41% in the purified dietary group and by 26% in the commercial and deficient dietary groups. Normal food consumption resumed in mice fed the commercial and purified diets after discontinuing drug therapy. In contrast, food intake remained depressed in tumor-bearing mice fed the deficient diet. Drug treatment did not alter food intake in any of the non-tumor-bearing groups. These data show that appetite suppression in the tumor-bearing groups is not related to drug toxicity.

Water intake was similar in commercial and purified dietary groups regardless of tumor, tumor type, or drug treatment and averaged 4.3 ± 0.1 (S.E.) ml. Water intake increased in mice fed the deficient diet during the 24-day stabilization period but decreased when their weight stabilized. Prestabilization intake was 11.6 ± 1.5 ml and decreased to 4.2 ± 0.1 ml at stabilization. Intake was unaffected by tumor, tumor type, or drug treatment.

Fast-growing Highly Pigmented Tumor Experiments and Diet Restriction Study. The effects of drug treatment and of food restriction on the median survival of mice implanted with the faster-growing, highly pigmented tumor are presented in Table 3 and Chart 2. Table 2 shows that the median survival for mice implanted with this tumor is 8 days less than that for mice implanted with the slow-growing tumor (Table 1). Mice maintained on the deficient diet again showed a significant increase in survival. Drug treatment increased survival in both the purified and deficient dietary groups. In general, the drug appears to be more effective against the faster-growing, more highly pigmented tumor in both purified and deficient dietary groups, and activity is enhanced by feeding the deficient diet. Drug treatment again was ineffective in mice maintained on the commercial diet (data not shown). Minimal or no increase in median survival resulted from food restriction.

Even though the food intake of the diet-restricted and the treatment groups were not different, the restricted groups lost more weight (Chart 2). The weight loss in these groups generally paralleled tumor-bearing groups given injections of the slow-growing tumor and treated with drug, except in mice on the purified diet. This group retained about 0.5 g more body weight than did their treated counterparts inoculated with the slow-growing tumor.

Protein (amino acid) and phenylalanine consumption before, during, and after the treatment interval for both drug-treated and food-restricted mice in all studies is compared in Tables 4 and 5. Table 4 illustrates that the mice maintained on the commercial and purified diets during the first 12 days after tumor inoculation were similarly divided into drug treatment (A) and the 0.9% NaCl solution-injected plus uninjected (•) subgroups; bars, S.E. Non-tumor-bearing mice contained 10 mice in the drug-injected subgroup and 20 mice in the 0.9% NaCl solution-injected plus uninjected subgroup. The number of tumor-bearing mice treated with drug were: Group A, 12; Group B, 16; and Group C, 16. The number receiving 0.9% NaCl solution or no injections were: Group A, 22; Group B, 26; and Group C, 22. The 0.9% NaCl solution-injected subgroup is combined with the uninjected group since they are not statistically different. Each non-tumor-bearing and tumor-bearing uninjected subgroup contained 10 mice. Drug treatment and 0.9% NaCl injections were given from Days 1 to 12. Arrows, first and last day of treatment.
Table 3
Effect of diet, drug treatment, and restricted feeding on survival and tumor weight in mice bearing the fast-growing B16 melanoma tumor

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group</th>
<th>Mean survival (days)</th>
<th>Median survival (days)</th>
<th>Increase in survival (%)</th>
<th>Mean tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified</td>
<td>Untreated</td>
<td>24 ± 1 †</td>
<td>23</td>
<td>9.0 ± 0.1 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>22 ± 1 †</td>
<td>24</td>
<td>6.4 ± 0.4 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>32 ± 2 †</td>
<td>31</td>
<td>5.7 ± 0.6 †</td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>Untreated</td>
<td>34 ± 2 †</td>
<td>30</td>
<td>3.3 ± 0.5 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>29 ± 3 †</td>
<td>30</td>
<td>3.4 ± 0.6 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>41 ± 3 †</td>
<td>41</td>
<td>3.6 ± 0.4 †</td>
<td></td>
</tr>
</tbody>
</table>

† Untreated and restricted groups received 0.9% NaCl solution and treated groups received drug from Days 1 to 12 after tumor inoculation on Day 0 as described in “Materials and Methods.” Actual food consumption was 1.7 ± 0.1 g from Days 1 to 12 and 2.6 ± 0.1 g thereafter in the purified diet-restricted group. Consumption was 1.9 ± 0.1 g in the deficient diet-restricted group throughout the experiment beginning on Day 1. Parallel consumption was observed in the drug-treated groups. Untreated and treated animals were given free access to the diets. Each group contained 10 mice.

% increase = \( \frac{\text{Median survival of group} - \text{Median survival of purified diet-untreated group}}{\text{Median survival of purified diet-untreated group}} \) × 100

³ Tumor weight determined at necropsy in animals that died from tumor, mean ± S.E.

² Mean ± S.E.

* Not significantly different from untreated group.

† Significantly different from untreated mice within dietary group (p < 0.001).

‡ Significantly different from all groups maintained on purified diet (p < 0.01) but not significantly different from each other.

The effect of the 3 diets on plasma tyrosine and phenylalanine levels is presented in Table 6. Plasma tyrosine levels in non-tumor-bearing mice fed the commercial and purified diets did not differ significantly. Phenylalanine levels were consistently higher in mice maintained on the commercial diet. Consumption of the deficient diet for 7 days resulted in a 33% decrease in plasma tyrosine and a 21% decrease in phenylalanine in non-tumor-bearing mice compared to the purified dietary group. These levels remain lowered for at least 70 days.

The presence of tumor significantly altered tyrosine and phenylalanine metabolism in mice fed the purified diet. Tyrosine levels increased by 9% 7 days after tumor transplant but then decreased by about 23% in mice bearing 14-day tumors. Plasma tyrosine was also reduced in mice fed the commercial and deficient diets, which bore 14-day tumors. Tumor-bearing animals maintained on the purified and deficient diets showed reduced plasma phenylalanine levels at 7 days with no further alteration in the levels at 14 days. Phenylalanine levels were also lower in 14-day tumor-bearing mice fed the commercial diet. Drug treatment did not alter plasma tyrosine and phenylalanine.

Tumor weight differed among the dietary groups. Tumors were weighed at necropsy after the mice died naturally from the tumor. These data are presented as part of Tables 1 and 3. Average tumor weights were not different in mice maintained on the commercial and purified diets as shown in Table 1. Tumor weights did not differ significantly between the drug-treated and untreated groups receiving the slow-growing tumor, but tumor weights were smaller in the treated group receiving the fast-growing tumor and maintained on the purified diet. Food restriction inhibited tumor growth in mice maintained on the purified diet and receiving the fast-growing tumor. Tumors were significantly smaller (p < 0.05) in mice maintained on the deficient diet than in any of the other dietary groups, but the tumors weighed the same in untreated, restricted, and treated groups regardless of tumor type.

* Manuscript in preparation.
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Table 4

Protein (amino acid) consumption in tumor-bearing mice during drug treatment and diet restriction

Protein (amino acid) consumption was determined daily for individual mice and averaged over the specified intervals. Initial consumption was determined over a 7-day period before tumor inoculation. Values for the treatment interval reflect the mean consumption during a 12-day drug treatment period or during food restriction. The posttreatment interval reflects the mean consumption during the 12 days after the treatment interval.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group (Tumor type)</th>
<th>Initial</th>
<th>Treatment interval</th>
<th>Posttreatment interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>Treated (slow)</td>
<td>35.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.7 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.9 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Purified</td>
<td>Treated (slow)</td>
<td>16.7 ± 0.2</td>
<td>9.6 ± 0.3</td>
<td>17.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>18.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.5 ± 0.2</td>
<td>17.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>16.3 ± 0.3</td>
<td>10.9 ± 0.3</td>
<td>16.2 ± 0.6</td>
</tr>
<tr>
<td>Deficient</td>
<td>Treated (slow)</td>
<td>18.4 ± 0.4</td>
<td>15.5 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19.2 ± 0.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>15.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>17.0 ± 0.2&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>17.7 ± 0.4</td>
<td>13.5 ± 0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14.2 ± 0.8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Consumption = Food consumption (g) during interval × % of protein in diet
<sup>b</sup> Values significantly different compared to initial consumption within the diet and group (p < 0.05).
<sup>c</sup> All values for commercial dietary group significantly different from all values for purified and deficient dietary groups (p < 0.05).
<sup>d</sup> Mean ± S.E.
<sup>e</sup> Significantly different from treated (slow) and restricted (fast) groups within diet (p < 0.05).
<sup>f</sup> All values significantly different from each other within the treatment interval (p < 0.05).

Table 5

Phenylalanine consumption in tumor-bearing mice during drug treatment and diet restriction

Phenylalanine consumption was determined daily for individual mice and averaged over the specified interval. Initial consumption was determined over a 7-day period before tumor inoculation. Values for the treatment interval reflect the mean consumption during the 12-day drug treatment period or during food restriction. The posttreatment interval reflects the mean consumption during the 12 days after the treatment interval. Values for tyrosine consumption (not shown) are equal to one-half those reported for phenylalanine.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group (tumor type)</th>
<th>Initial</th>
<th>Treatment interval</th>
<th>Posttreatment interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>Treated (slow)</td>
<td>1621 ± 20&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1259 ± 43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1814 ± 45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Purified</td>
<td>Treated (slow)</td>
<td>847 ± 12</td>
<td>488 ± 17&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>867 ± 21</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>940 ± 7</td>
<td>535 ± 10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>950 ± 12</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>827 ± 15</td>
<td>553 ± 13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>825 ± 31</td>
</tr>
<tr>
<td>Deficient</td>
<td>Treated (slow)</td>
<td>125 ± 2</td>
<td>105 ± 4</td>
<td>130 ± 5</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>104 ± 1</td>
<td>77 ± 1</td>
<td>115 ± 1</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>120 ± 3</td>
<td>91 ± 2</td>
<td>96 ± 5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Consumption = Food consumption (g) during interval × % of phenylalanine in diet
<sup>b</sup> All values for commercial dietary group significantly different from all values for purified and deficient groups. Values within dietary groups significantly different from each other (p < 0.05).
<sup>c</sup> Mean ± S.E.
<sup>d</sup> Values significantly different from initial and posttreatment interval (p < 0.05).
<sup>e</sup> Significantly different from treated (fast) and restricted (fast) group during treatment interval (p < 0.05).
<sup>f</sup> Values from all intervals significantly different from treated (slow) and restricted (fast) groups (p < 0.05).
<sup>g</sup> All values significantly different from all other dietary groups during all intervals (p < 0.05).

Mice showed no visible lung metastasis when observed at necropsy in any of the groups. The mice may have been killed by the primary tumor before evidence of pulmonary metastasis could be detected.

DISCUSSION

In this study, we examined the concomitant use of dietary limitation of tyrosine and phenylalanine and of carbidopa-levodopa methyl ester chemotherapy in the treatment of B16 melanoma. The results indicate that host survival is enhanced with the deficient diet-drug combination. The data establish dietary phenylalanine and tyrosine as important variables in determining the chemotherapeutic responsiveness to this drug and further support a role for tyrosine and phenylalanine in melanoma growth.

Consideration of diet would be particularly important if this drug or perhaps other analogs become clinically useful in the treatment of human melanoma patients. One way of insuring control over tyrosine and phenylalanine levels would be through providing the patient with parenteral nutritional support restricted in tyrosine and phenylalanine content. Conventional
parenteral nutritional support could interfere with the therapeutic response to levodopa therapy since the currently available protein hydrolysates and crystalline amino acid preparations contain high amounts of phenylalanine (40). In fact, blood levels of phenylalanine and other amino acids are elevated during infusion of these amino acid-containing solutions (40).

In our study, we observed a poor chemotherapeutic response in mice maintained on the commercial diet, which contains 1.09% phenylalanine and 0.64% tyrosine. The antitumor response to drug also is inhibited in mice that are maintained on a diet similar to the purified diet but containing 2% phenylalanine and 1% tyrosine. Although these data support dietary phenylalanine and tyrosine as important modifiers of the drug response, interference by other constituents cannot be ruled out. The fact that survival of tumor-bearing mice maintained on the commercial diet was longer than survival of mice maintained on the purified diet suggests that the commercial diet contains some tumor-inhibitory constituent, which may also interfere with drug activity.

Weight loss as a side effect of drug treatment occurred in all dietary groups. Patients with Parkinson's disease treated with these drugs also show weight loss, which may be due to appetite suppression (38) or to effects on metabolic rate (32). We observed no depression of appetite in non-tumor-bearing, drug-treated mice, but appetite was depressed in tumor-bearing animals. Both groups still lost weight during drug treatment. These data suggest that the anorexia in tumor-bearing animals is related to the tumor and not to the drug treatment. From the diet restriction study, it is clear that decreased food consumption and the accompanying weight loss does not significantly alter survival of tumor-bearing mice. Feeding mice the 23.2% protein equivalent diet, which eliminates protein-calorie malnutrition as a contributing factor to the antitumor effect during drug treatment, does not alter median survival compared to treated mice maintained on the purified diet.

In contrast to the complete depletion of asparagine that is required for asparagine-sensitive tumors, only partial reductions in the plasma level of tyrosine and phenylalanine are necessary to achieve an antitumor response in the B16 melanoma tumor. Only minimal weight loss occurred in mice fed the deficient diet, and their weight was stable before tumor inoculation. The antitumor effect derived from this diet is clearly related to weight loss but probably is related to the plasma level of tyrosine and phenylalanine available to the tumor. These data further support an altered nutritional requirement for tyrosine and phenylalanine in melanoma tumors.

Additional studies are in progress to determine the specific levels of tyrosine and phenylalanine required for the growth of B16 melanoma in vivo, to determine the extent of inhibition imposed by dietary phenylalanine and tyrosine on levodopa uptake into melanoma cells, and to establish the mechanism responsible for inhibition of tumor growth by tyrosine and phenylalanine restriction.

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Dietary Influence of Tyrosine and Phenylalanine on the Response of B16 Melanoma to Carbidopa-Levodopa Methyl Ester Chemotherapy


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