dietary influence of tyrosine and phenylalanine on the response of B16 melanoma to carbidopa-levodopa methyl ester chemotherapy

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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 toymycin and 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 F, (hereafter called B6D2F) 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 antimelanoma 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 24 ± 1° and humidity was controlled to 53 ± 5%. There were 24 to 30 mice per cage. Water intake was 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 4.5% crude fiber and provides 3.86 kcal/g of food. This diet also contained 0.015% 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 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 B6D2F mice.2 The 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 Bounous and Kongshavn (3) and by Theuer (36). The diet contained 0.65% L-phenylalanine, 0.33% L-tyrosine, 0.50% L-isoleucine, 0.8% L-leucine, 0.9% L-lysine, 0.43% 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 carbohydrate, and 5% cellulose as a source of fiber. Five % HWM 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, 458; 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, 0.88; 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 minerals (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-inositol, 5; choline chloride, 75; menadione, 2.25; p-amino benzoic 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.

Dietary and Levodopa Methyl Ester Therapy

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 24 hr following tumor inoculation and continuing for 12 days. Carbidopa (100 mg/kg) was administered 90 min prior to injection of levodopa
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 access to the diets and were handled as outlined under "Materials and Methods." Beginning on Day 0, food intake was limited to 1.6 ± 0.1 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 drug-treated mice bearing the slow-growing tumor (Table 2). Both restricted subgroups were given injections of 0.9% NaCl solution from Day 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-

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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*</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*</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*</td>
</tr>
<tr>
<td></td>
<td>Treated (16)</td>
<td>53 ± 2*</td>
<td>50</td>
<td>61</td>
<td>3.4 ± 0.6*</td>
</tr>
</tbody>
</table>

* 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."

b % of increase = \[
\frac{\text{Median survival of dietary untreated or treated group} - \text{Median survival of purified diet-untreated group}}{\text{Median survival of purified diet-untreated group}} \times 100
\]

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

d Numbers in parentheses, number of mice in each group.

e Mean ± S.E.

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

g 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**

Food consumption was determined daily and averaged over the specified periods. Initial consumption was determined over a 7-day period before tumor inoculation and/or drug injection. Values for consumption during the 12-day treatment period and during the 10-day posttreatment period in tumor-bearing and non-tumor-bearing mice receiving daily i.p. injections of drug are presented. Each purified and deficient dietary tumor-bearing group contained 16 mice, and the commercial dietary group contained 12 mice. All non-tumor-bearing groups contained 10 mice.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Mean food consumption (g)</th>
<th>Treatment period</th>
<th>Posttreatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Tumor*</td>
<td>Nontumor</td>
</tr>
<tr>
<td>Commercial</td>
<td>3.4 ± 0.1*</td>
<td>2.5 ± 0.1</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Purified</td>
<td>2.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Deficient</td>
<td>2.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>

* Significantly different from initial food consumption within dietary group (p < 0.001).

Significantly different from commercial diet group (p < 0.001).
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 14-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 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
all subgroups contained 10 mice. Arrows, first and last days of treatment.

diets, consumption decreased in the treated and restricted diets consumed similar amounts of amino acids. In all commercial diet clearly were not malnourished for protein. Non-tumor-bearing mice maintained on the purified and deficient diets consumed similar amounts of amino acids. In all diets, consumption decreased in the treated and restricted groups during the treatment interval. Mice maintained on the deficient diet actually consumed more amino acids on a mg/kg basis than did mice maintained on the purified diet during the treatment interval. The diet restriction study shows that the decrease in amino acid intake in the purified and deficient dietary groups does not increase median survival (Table 3).

Additional studies in our laboratory indicate that increasing the protein equivalent of the purified diet from 11.8 to 23.2% by doubling the amounts of all amino acids except for phenylalanine and tyrosine does not alter survival or the antitumor response to drug. Even though amino acid intake was doubled, mice still lost a similar amount of weight when treated with the drug.

Table 5 illustrates that an average phenylalanine consumption ranging from 77 to 130 mg/kg increases median survival (deficient diet). Drug treatment decreases phenylalanine (and tyrosine) consumption further, but these changes are not statistically different (p > 0.05). Other experiments indicate that the phenylalanine intake must be lower than 364 ± 10 mg/kg before an increase in median survival occurs. This value is significantly lower than is the 488 ± 17 mg/kg consumption observed in the slow-growing tumor-bearing group maintained on the purified diet (p < 0.05).

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.
Dietary and Levodopa Methyl Ester Therapy

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 ± 0.5d</td>
<td>27.7 ± 0.9d</td>
<td>39.9 ± 1.0d</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 ± 0.1e</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 ± 0.6f</td>
<td>19.2 ± 0.7f</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>15.4 ± 0.1e</td>
<td>11.4 ± 0.1f</td>
<td>17.0 ± 0.2f</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>17.7 ± 0.4</td>
<td>13.5 ± 0.4g</td>
<td>14.2 ± 0.8g</td>
</tr>
</tbody>
</table>

※ Food consumption (g) during interval
※※ All values for commercial dietary group significantly different from all values for purified and deficient dietary groups (p < 0.05).
※№ Mean ± S.E.
※∥ Values significantly different from initial and posttreatment interval (p < 0.05).
※' Values significantly different from treated (slow) and restricted (fast) groups within diet (p < 0.05).
※$ Values significantly different from each other within the treatment interval (p < 0.05).
※% Values significantly different from initial and posttreatment interval (p < 0.05).
※(|) Values significantly different from treated (slow) and restricted (fast) groups during treatment interval (p < 0.05).
※(|) All values significantly different from all other dietary groups during all intervals (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 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</td>
<td>1259 ± 43</td>
<td>1814 ± 45</td>
</tr>
<tr>
<td>Purified</td>
<td>Treated (slow)</td>
<td>847 ± 12</td>
<td>488 ± 17</td>
<td>867 ± 21</td>
</tr>
<tr>
<td></td>
<td>Treated (fast)</td>
<td>940 ± 7</td>
<td>535 ± 10</td>
<td>950 ± 12</td>
</tr>
<tr>
<td></td>
<td>Restricted (fast)</td>
<td>827 ± 15</td>
<td>553 ± 13</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>

※ Consumption = ——— × % of protein in diet
Weight (g) during interval
※※ All values for commercial dietary group significantly different from all values for purified and deficient dietary groups (p < 0.05).
※№ Mean ± S.E.
※∥ Values significantly different from initial and posttreatment interval (p < 0.05).
※$ Values significantly different from initial and posttreatment interval (p < 0.05).
※(|) Values significantly different from treated (slow) and restricted (fast) groups during treatment interval (p < 0.05).
※(|) 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 was 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 antimalanoma 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 not 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.

ACKNOWLEDGMENTS

The authors are grateful to Judith Salmon and Kai Johnson for their expert technical assistance and to Dr. Craig Coon for his helpful comments regarding formulation of the chemically defined diets.

REFERENCES


Table 6

Effect of diet, tumor, and drug treatment on plasma tyrosine and phenylalanine

<table>
<thead>
<tr>
<th>Time on diet</th>
<th>Time with tumor</th>
<th>Tyrosine</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial diet</td>
<td>21</td>
<td>68 ± 3 (6)</td>
<td>59 ± 0 (2)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>59 ± 1 (6)</td>
<td>60 ± 0 (2)</td>
</tr>
<tr>
<td>Purified diet</td>
<td>21</td>
<td>66 ± 2 (9)</td>
<td>61 ± 1 (9)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>72 ± 2 (6)</td>
<td>53 ± 1 (6)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>51 ± 3 (2)</td>
<td>49 ± 0 (2)</td>
</tr>
<tr>
<td>Deficient diet</td>
<td>7</td>
<td>44 ± 4 (6)</td>
<td>48 ± 2 (6)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>45 ± 3 (6)</td>
<td>51 ± 3 (6)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>46 ± 1 (5)</td>
<td>37 ± 1 (5)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>31 ± 1 (1)</td>
<td>43 ± 1 (1)</td>
</tr>
</tbody>
</table>

* Tyrosine and phenylalanine levels were determined on pooled plasma samples obtained from groups of 4 to 6 mice for the total number of pooled samples analyzed.
* Mean ± S.E.
* Numbers in parentheses, number of samples analyzed.
* Purified diet non-tumor-bearing untreated versus commercial diet non-tumor-bearing untreated (p < 0.001).
* Purified diet tumor-bearing untreated versus non-tumor-bearing untreated (p < 0.001).
* Significantly different from purified diet tumor-bearing untreated group with 7-day tumor.
* All values significantly different from commercial and purified diet tumor-bearing and non-tumor-bearing mice (p < 0.001).
* Deficient diet tumor-bearing versus non-tumor-bearing (p < 0.001).
Dietary and Levodopa Methyl Ester Therapy

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


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