Effects of Branched Chain Amino-acid-enriched Total Parenteral Nutrition on Amino Acid Utilization in Rats Bearing Yoshida Sarcoma

Lisa E. Crosby, Bruce R. Bistrian, Pei-ra Ling, Nawfal W. Istfan, George L. Blackburn, and Stacy B. Hoffman

ABSTRACT

We have studied the ability of branched chain amino-acid enriched total parenteral nutrition solutions to improve nutritional status without stimulating tumor growth. Protein kinetics, fractional synthetic rates of individual tissues, and albumin synthesis were compared in male Sprague-Dawley rats (125-145 g) that had either s.c. Yoshida sarcoma (n = 15) or sham implantations (n = 18). Ten days postinjection, rats were randomly assigned to 2 diet groups and given parenteral infusions of 4 days at 170 kcal/kg-body wt-day as dextrose and 2 g N/kg-body wt-day as either 19 or 50% branched chain amino acid-enriched diet. During the last 4 h of feeding, protein kinetic values were studied using a constant infusion of [14C]tyrosine. Plasma tyrosine appearance, synthesis, and breakdown were unchanged by branched chain amino acid infusion. Percentage of tyrosine flux oxidized and tyrosine oxidation decreased (P < 0.05) and net tyrosine balance improved (P < 0.05) in rats receiving the branched chain amino acid-enriched diet. Greater nitrogen balance and lower tumor growth rates were also found in branched chain amino acid-infused rats although not statistically significant. Tumor intracellular specific activity was significantly higher in tumor animals receiving crystalline infusions, suggesting greater tumor protein breakdown with branched chain amino acid-enriched infusion. Fractional synthetic rates of liver, muscle, and tumor were unchanged. Hence, branched chain amino acid-enriched total parenteral nutrition increases amino acid utilization for net protein synthesis principally by reducing oxidation without stimulating tumor growth.

INTRODUCTION

Cancer cachexia is recognized as a primary cause of death in many types of cancer (1). In fact, protein malnutrition is the single most common secondary diagnosis in cancer patients (2). Failure to prevent protein malnutrition increases the risk of developing complications, aggravates antineoplastic therapy, reduces the quality of life, and increases morbidity and mortality (2-5).

Although malnutrition may result from interference with gastrointestinal function, the usual cachexic syndrome developing in the course of malignant disease often cannot be explained by depression of food intake due to local factors alone and behaves as if it were the result of a systemic alteration including anorexia induced by the tumor (6). Furthermore, the weight loss accompanying cancer in humans bears no consistent relationship with the type, duration, site, size, or number of metastases (7).

Recent evidence indicates a similarity between the metabolic response to injury and active tumor growth (8). This similarity raises interest in a possible role for BCAA in modifying the response to injury and active tumor growth (8). This similarity has been recognized in the treatment of cancer cachexia. On day 10, the tumor-bearing animals weighed less rapidly growing tumors had diminished food intake, reflecting the presence of tumor. On day 10, the tumor-bearing animals weighed less than their cage mates and were not pair fed. The animals were weighed, returned to their cages, and allowed to consume standard laboratory chow and tap water ad libitum.

The Yoshida sarcoma tumor cell line was used to induce cancer cachexia. The study was conducted from day 10 to 14 which is on the linear portion of the tumor growth curve and was determined to be a cachectic period (Fig. 1). On day 0 of the study, 15 animals were inoculated with 10^5 cells of viable Yoshida sarcoma into the s.c. area of the right flank. The non-tumor-bearing rats were given an identical sham injection with sterile saline. The animals were weighed, returned to their cages, and allowed to consume standard laboratory chow and tap water ad libitum.

Streuli and his associates demonstrated that continuous p.o. administration of BCAA to rats bearing Yoshida sarcoma significantly increased survival time by 32% and reduced tumor size by 33% after 3 wk of growth (17). Their work also demonstrated that a shift in nitrogen balances was delayed, but not prevented, by BCAA administration (17). In our laboratory, Kawamura et al. found that BCAA-enriched solutions infused into animals bearing RNC-fibrosarcoma limited the reduction in skeletal protein synthesis seen in tumor-bearing animals receiving standard amino acid formulas (18). Also, Tayek and his coworkers gave parenteral infusions containing 50% BCAA-enriched solutions to malnourished patients with intraabdominal metastatic adenocarcinoma and improved whole body leucine flux, oxidation, leucine balance, and fractional synthetic rates of albumin (19). However, there was concern in the latter study that the choice of leucine as the tracer when leucine-enriched solutions are studied might lead to underestimates of leucine oxidation and therefore overestimates of leucine balance. Thus, we sought to confirm the benefits of parenterally administered BCAA for kinetic estimates of protein metabolism using tyrosine as the amino acid tracer. Since the tyrosine content of both formulas is very low and lower in the BCAA-enriched solution, a finding of improvement in parameters of protein metabolism with the BCAA-enriched solution using tyrosine as the tracer amino acid is consistent with previous findings in other tumor models (5-7).

It is important to note that in the latter study the choice of leucine as the tracer when leucine-enriched solutions are studied might lead to underestimates of leucine oxidation and therefore overestimates of leucine balance. Thus, we sought to confirm the benefits of parenterally administered BCAA for kinetic estimates of protein metabolism using tyrosine as the amino acid tracer. Since the tyrosine content of both formulas is very low and lower in the BCAA-enriched solution, a finding of improvement in parameters of protein metabolism with the BCAA-enriched solution using tyrosine as the tracer amino acid is consistent with previous findings in other tumor models (5-7).
than the non-tumor-bearing animals, indicating their cachectic state. The rats were anesthetized with ether and a Silastic catheter (0.025 inside diameter x 0.047 in outside diameter; Dow-Corning Laboratories, Corning, NY) was inserted through the internal jugular vein as previously described (20). The animals were placed in metabolic units and i.v. infused with saline (approximately 20 ml of sodium chloride (154 mM) overnight from a Holter pump (Critikon Inc., Tampa, FL)). On day 11, the animals were randomly assigned to 2 groups and i.v. administered diets containing amino acids and one half the dextrose calories (Table 1) to allow adaptation to the glucose. There was no significant difference in the size of the tumors, as indicated by measurements of tumor volume, between the two diet groups. The animals were given the full calorie diet on day 12 and feeding continued for an additional 2 days. At the full calorie level both diets contained 220 calories (Table 1) to allow adaptation to the glucose. There was no significant difference in the size of the tumors, as indicated by measurements of tumor volume, between the two diet groups. The animals were given the full calorie diet on day 12 and feeding continued for an additional 2 days. At the full calorie level both diets contained 220 calories/kg-body wt • day, of which 75% was hydrous dextrose and 25% was amino acids (2 g amino N/kg-body wt • day as either 19% BCAA or 50% BCAA (Table 1). The diets were isonitrogenous, isovolemic, and isocaloric and were formulated in the hospital pharmacy under aseptic conditions. An infusion of 40 ml per day was sufficient to deliver the total amount of calories. The animals were infused for 3.5 days which has been demonstrated to be a sufficient time to reveal differences if they indeed exist. Hyperalimentation in a small tumor-bearing rat for a longer period of time (i.e., 10–14 days) is difficult to accomplish due to technical failures and an increase in mortality. Twenty four-h urine was collected for nitrogen balance determination.

Isotopic Turnover Design. On day 14, ["C"]tyrosine (350 mCi/mmol; ICN, Irvine, CA) was added to the diets and a 4-h constant infusion was conducted to investigate protein kinetics. Each animal received 1.7 µCi/h of ["C"]tyrosine at a volume rate of 1.67 ml/h through a syringe pump (±1%; Harvard Apparatus Co., S. Natick, MA).

Total carbon dioxide production, O2 consumption, respiratory quotient, and resting energy expenditure were determined during the infusion as previously described (20). VO2 and VCO2 were measured by methods previously published by our laboratory (20).

At the end of the infusion, the animals were sacrificed by decapitation and the blood was collected in heparinized tubes. Plasma was separated by centrifugation and stored at −25°C. Immediately following decapitation, the body was quickly dissected, and the liver, tumor, and portions of the abdominus rectus muscle were removed. The liver was weighed and 1-g pieces were placed in 5 ml of 10% sulfosalicylic acid and 5 ml of saline and then completely frozen in liquid nitrogen (−180°C) to halt all metabolic processes. Two 1-g pieces of muscle were similarly frozen. The tumor was weighed and also frozen intact in liquid nitrogen. All samples were stored at −25°C until analysis. The total time between decapitation and sample freezing in liquid nitrogen was 2–3 min.

Analytical Methods. VO2, VCO2, respiratory quotient, and resting energy expenditure were derived from the equations of Weir (21).

Total urinary nitrogen was determined following a micro-Kjeldahl digestion.

In preparation for the tyrosine and albumin specific activity assays, the plasma was thawed and deproteinized with 10% trichloroacetic acid (2:1 v/v; plasma:trichloroacetic acid). Following centrifugation, the supernatant was analyzed for free tyrosine specific activity as previously described (22). Tyramine concentration was determined by the method of Waalkes and Undenfriend (23).

The protein pellet was analyzed for albumin specific activity by means of an ethanol-water extraction. One ml of the extracted supernatant was added to 10 ml of commercial scintillant (Monosfluor; National Diagnostics, Manville, NJ) with 100 µl of glacial acetic acid to suppress chemiluminescence and analyzed, in duplicate, for ["C"]radioactivity (LS-8000 spectrometer; Beckman, Fullerton, CA). To determine albumin concentration, 25 µl of the ethanol supernatant were transferred, in duplicate, into test tubes containing 6 ml of a bromoresol green solution (carbon substrate; General Diagnostics, Morris Plains, NJ) and distilled water (1:5 v/v solution) and vortexed for 10 sec. The samples were analyzed spectrophotometrically at a wavelength of 630 nm. Albumin concentrations were determined by linear regression of known standards, and unknowns were determined from the regression line.

The tissue samples, liver, abdominus rectus muscle, and tumor were thawed and homogenized (Polytron; Brinkmann Instruments, Westbury, NY). Nitrogen content of tissue samples stored in saline was determined by micro-Kjeldahl digestion. Homogenized tissue samples stored in sulfosalicylic acid were centrifuged at 4500 rpm to separate the acid-soluble amino acids (SI) from protein-bound precipitate (SB). The acid-soluble amino acid supernatant for each sample was analyzed for tyrosine specific activity as stated previously. The protein-bound precipitate was washed with sulfosalicylic acid and recentrifuged. The protein pellet was then dried at 100°C.

To determine the amount of tyrosine in the protein-bound precipitate, 200-µg samples of wet tissue (liver, muscle, and tumor) were obtained from noninfused animals. These samples were hydrolyzed in 10 ml of 6 N HCl at 115°C for 24 h. Tyrosine concentration (µmol tyrosine/mg protein) of the hydrolysate was determined on a reverse-phase column (C18 Bondapack; Waters Associates, Milford, MA) by high performance liquid chromatography using gradient elution with phosphate buffer and 65% methanol and precolumn derivitization with 0-phthalaldehyde and fluorescence detection. Nitrogen content (N/µg) of 40 µg of dry protein-bound precipitate was determined by micro-Kjeldahl digestion. Two ml of Tissue Solubilizer-450 (Beckman Instruments, Inc., Fullerton, CA) were added to another 40-µg aliquot of
dried protein-bound precipitate and the samples were incubated over-
night at 50°C. Ten ml of commercial scintillant (Beckman NA) were
added to the vials with 200 μl of glacial acetic acid to suppress
chemiluminescence and analyzed for [14C] radioactivity (dpm/mg) (Beck-
man LS-8000 spectrometer).

Rate of whole-body tyrosine appearance, oxidation, percentage of
tyrosine oxidized, synthesis, breakdown, and tyrosine balance were
estimated from the equations of Waterlow (24). It was assumed that a
plateau labeling (steady state) of the plasma compartment was achieved
when the Smean was reached in the expired breath (between 2 to 3 h of
continuous [14C]tyrosine infusion) (25). Plasma tyrosine appearance
can be attributed to the degradation of body protein, dietary intake, or
hydroxylation of phenylalanine (Fig. 1). It was previously shown in our
laboratory that approximately 22% of tyrosine flux is derived from
phenylalanine (26). Studies done by other investigators (27) have shown
similar proportions of phenylalanine-derived plasma tyrosine, suggest-
ing that this fraction is relatively stable in animals with normal liver
function. In this experiment, most of the plasma phenylalanine flux is
derived from protein breakdown since dietary intake was negligible
(Table 1). Under these conditions, it can be shown that a 50% increase
in the rate of conversion to tyrosine will result in only a 10% increase
in tyrosine appearance in excess of what is derived from breakdown.
Therefore, it is appropriate to consider that changes in plasma tyrosine
appearance in this study are closely related to changes in whole-body
protein breakdown. The protein fractional synthetic rates in the liver,
abdominus rectus muscle, and tumor were derived from the equations
of Garlick et al. (28).

Estimates of fractional tumor growth, Kg, were derived from tumor
volume measurements on days 10 and 14. Tumor volumes were esti-
mated from measurements of tumor length, width, and depth in milli-
imeters as previously described (29). These measurements bear a close
relationship to tumor weight. Tumor protein breakdown rates were
estimated as the difference between tumor protein synthesis, measured
isotopically, and tumor growth. Therefore, Kg is expressed as the rate of
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Statistical Analysis. Data are presented as mean ± SEM. The data
from groups one through four were compared for statistical differences
using two-way and one-way analysis of variance using statistical soft-
ware (BMDP Statistical Software, Los Angeles, CA).

RESULTS

During a constant infusion of L-[U-14C]tyrosine, radioisotope
excretion in expired breath generally reached a steady state
within 2–4 h. Whole-body tyrosine kinetic values are shown in
Table 2. There was no significant difference in mean whole-
body protein flux due to BCAA infusion. The use of BCAA-
enriched diets significantly reduced whole-body tyrosine oxida-
tion and percentage of flux oxidized compared to animals

<table>
<thead>
<tr>
<th>Table 2 Whole-body tyrosine kinetics</th>
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<tr>
<td><strong>Tumor</strong></td>
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<tr>
<td>BCAA (7)</td>
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<tr>
<td>Flux</td>
</tr>
<tr>
<td>% of flux oxidized</td>
</tr>
<tr>
<td>Oxidation</td>
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<td>Synthesis</td>
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<td>Breakdown</td>
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<td>Tyrosine balance</td>
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* Mean ± SEM (μmol/h/g body weight). P < 0.05 two-way analysis of variance (BCAA versus CAA).
* P < 0.05 one-way analysis of variance (BCAA versus CAA).

Nitrogen analysis revealed significantly higher nitrogen bal-
ance in tumor-bearing rats versus the non-tumor controls (Fig.
2). The greater nitrogen retention in the tumor-bearing animals
may be due to the tumor functioning as a “nitrogen trap” (29)
particularly as the animals were studied during a rapid tumor
growth phase. Also, the nitrogen balance of both the tumor and
non-tumor-bearing animals infused with BCAA-enriched solu-
tions was numerically higher than those infused with crystalline
solutions.

All of the animals in the study lost weight, but there were no
significant differences in final body weight or weight change.
The weight loss may be explained by the small size of the
animals, the effects of surgical stress, and the administration of
only half of the dextrose calories the first night of feeding to
allow for adaptation to parenteral glucose.

Tumor measurements and kinetics are displayed in Table 3.
There were no significant differences in tumor volume, weight,
percentage of body weight, or fractional synthetic rates due to
diet manipulation. The growth rate of the Yoshida sarcoma, as
indicated by measurements of tumor volume, was decreased to
an insignificant degree by BCAA infusion. Tumor breakdown
rates, determined by the difference between tumor synthesis
and tumor growth, were higher but also not significant in the
animals receiving BCAA-enriched infusions. Of note, however,
tumor intracellular specific activity was significantly higher
(P < 0.05) in animals infused with crystalline amino acids which
is consistent with greater tumor breakdown with BCAA infu-
sion.

The rates of fractional protein synthesis were calculated using
the specific radioactivities of the plasma and the protein bound
precipitate. No change in the synthetic rates of liver, rectus

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Fig. 2. Nitrogen balance. Nitrogen balance was determined to be significantly
higher in tumor-bearing animals as compared to non-tumor-bearing animals. The
animals infused with BCAA-enriched solutions were in a more positive state than
those infused with crystalline solutions, but this did not reach a level of statistical
significance. Mean ± SEM, P < 0.05 tumor versus nontumor.

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2700
There were no significant differences in tumor volume, weight, percentage as body weight, or fractional synthetic rate due to diet manipulation. Growth rate as indicated by measurements of tumor volume was decreased, but not at a significant level. Tumor protein breakdown rates (fractional synthetic rate - growth rate) increased in rats infused with BCAA-enriched diets, but not significantly. Intracellular specific activity (normalized for total infusate volume) was significantly lower in rats receiving BCAA infusions suggesting greater tumor protein breakdown with BCAA-enriched infusions.

**DISCUSSION**

Cancer-associated cachexia is the primary cause or an associated phenomenon in many tumor-related deaths (1). Early experience suggested that poor nutrition in cancer patients interfered with therapy similar to the situation found in acute or chronic nonmalignant illness, and that malnutrition could interfere with the delivery of the optimal oncological therapy (31). Moreover, other evidence had been gathered that nutritional repletion of the malnourished cancer patient with standard regimens was difficult, if not impossible (32). In considering the consequences of protein malnutrition in the tumor-bearing host, we sought to develop a more effective nutritional support therapy through the use of BCAA.

In patients with actively growing neoplasms, plasma amino acid profiles are characterized by reduced BCAA levels and arterial-venous differences across skeletal muscle reflect an increased proportion of BCAA being oxidized (33). BCAA administered p.o. were shown to increase survival time and nitrogen balance while reducing tumor growth (17). There is also evidence for a beneficial effect of BCAA-enriched infusion on plasma leucine and albumin kinetics in humans (19) and muscle protein turnover in animals with cancer (18).

This study examined the effects of BCAA on host protein synthesis, breakdown, and growth. Although tyrosine is not an essential amino acid and rates of tyrosine release from protein breakdown cannot be derived from its appearance without additional assumptions, the rate of tyrosine oxidation and incorporation into protein can be evaluated (34). There was a significant reduction in whole-body tyrosine oxidation and percentage of tyrosine flux oxidized in the animals given BCAA infusions. Also, with the reasonable assumption that tyrosine appearance by de novo synthesis from phenylalanine does not differ between the two diet groups, net tyrosine balance was significantly greater in animals infused with BCAA-enriched solutions. Even without this assumption, the de novo synthesis of tyrosine is very small in relation to tyrosine appearance from breakdown and is unlikely to seriously affect the tyrosine balance calculation.

In injury and sepsis, the metabolic response is characterized by the net degradation of muscle protein and the mobilization of body fat for energy substrates and gluconeogenesis (35). BCAA are preferentially utilized in skeletal muscle and it has been hypothesized that BCAA supplementation may provide a protein-sparing metabolic fuel when energy demands increase due to the metabolic response to stress or disease. Freund et al. hypothesized that BCAA spared nitrogen by providing substrates for muscle fuel catabolism, by providing amino groups to produce alanine for hepatic gluconeogenesis, and by blocking amino acid efflux from muscle (36). Although the metabolic response in a tumor model is somewhat different from an injury or trauma model, there are numerous similarities (8) and these principles of BCAA utilization may apply in the tumor-bearing host. Administration of TPN solutions enriched with BCAA reduces whole-body tyrosine oxidation and percentage of tyrosine flux oxidized and spares body protein by improving amino acid reutilization through nonoxidative pathways. Thus, such a dietary manipulation may favorably influence net protein metabolism in the carcass without enhancing tumor growth.

The tumor-bearing animals retained more nitrogen than the non-tumor-bearing animals (P < 0.05). This significant difference may be due to the tumor functioning as a nitrogen trap where tumor breakdown is minimal, limiting systemic reentry of amino acids.

**REFERENCES**

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- The tumor-bearing animals retained more nitrogen than the non-tumor-bearing animals (P < 0.05). This significant difference may be due to the tumor functioning as a nitrogen trap where tumor breakdown is minimal, limiting systemic reentry of amino acids.
Thus, the data suggest that administration of TPN solutions enriched with BCAA does not stimulate tumor growth but does support host protein metabolism and may play a role in retarding tumor growth by increasing tumor protein breakdown rates.

In conclusion, our results suggest that BCAA-enriched TPN solutions increase amino acid utilization for net protein synthesis by the host principally by reducing oxidation without stimulating tumor growth. The site of the improved protein balance was not specifically identified but presumably was in skeletal muscle due to the large size of this component as well as being the usual major site of net nitrogen balance during feeding. Such a location would also be consistent with the mode of action of BCAA to reduce net skeletal protein catabolism. These observations may be important for the development of optimal nutritional support therapies for cancer patients in the treatment of cancer cachexia. Improved support regimens might afford a heretofore undetected benefit of nutritional support in randomized trials of cancer patients receiving chemotherapy (37) and might also be an effective means to slow the progression of the cachexia syndrome and prolong life where effective chemotherapy does not exist. Finally, the suggestive effect of BCAA-enriched total parenteral nutrition to increase tumor breakdown rates deserves further investigation over a longer study period.

REFERENCES

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