Glutamine and Arginine Metabolism in Tumor-Bearing Rats Receiving Total Parenteral Nutrition

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Arginine supplementation increases glutamine levels in muscle and plasma. Since glutamine production is increased in catabolic states, these observations prompted us to investigate whether the flux of arginine to glutamine was increased in tumor-bearing (TB) rats, and we measured the synthesis rate of glutamine from arginine in control versus TB rats receiving standard total parenteral nutrition (TPN) solution. Male Donryu rats (N = 36; body weight, 200 to 225 g) were divided into two groups, control and TB rats. Yoshida sarcoma cells (1×10^6) were inoculated into the back of the rats (n = 18) subcutaneously on day 0. The rats were given free access to water and rat chow. On day 5, all animals, including non-TB rats (n = 18), were catheterized at the jugular vein and TPN was begun. On day 10, TPN solution containing either U-^{14}C-glutamine (2.0 μCi/h) or U-^{14}C-arginine (2.0 μCi/h) was infused as a 6-hour constant infusion. At the end of the isotope infusion, plasma was collected to determine the glutamine production rate in rats receiving U-^{14}C-glutamine, and the ratio of specific activity of glutamine to specific activity of arginine was measured in rats receiving U-^{14}C-arginine. Only 2 g tumor caused a decrease in glutamine levels and an increase in glutamine and arginine production. The low flux rate of arginine to glutamine was observed in control rats (Arg to Gln, 41.0 ± 11.9 μmol/kg/h). On the other hand, TB caused a significant increase in Arg to Gln compared with the control (213.3 ± 66.1 μmol/kg/h, $P < .01$ *v* control). An increase in the flux rate of Arg to Gln was associated with an enhancement in the ratio of specific activity of ornithine to specific activity of arginine in TB rats (control 51.5% ± 10.9% *v* 77.4% ± 8.9%, $P < .05$). We conclude that (1) glutamine and arginine metabolism is altered with very small tumors, (2) although the flux of Arg to Gln was increased in TB and rats, the small increase in Arg to Gln cannot explain the observed large increase in Gln production.

PREVIOUS REPORTS by Ardawi[1] and our group[2] have shown that glutamine production is increased in burned and septic rats. Glutamine is derived from proteolysis and is synthesized from other amino acids in skeletal muscle under surgical stress. The branched-chain amino acids (BCAA) leucine, isoleucine, and valine provide the amino nitrogen for glutamine synthesis via transamination in skeletal muscle.[3] On the other hand, it is believed that the source of the carbon skeleton for glutamine synthesis is acetyl coenzyme A or pyruvate derived from glycolysis.[3] Since glucose uptake in peripheral tissues is impaired due to insulin resistance, incorporation of the carbon skeleton of amino acids into glutamine is increased in catabolic states. Indeed, we have previously demonstrated that glutamine synthesis derived from leucine carbon is increased in septic rats.[2]

We have also shown that glutamine supplementation prevented a reduction of glutamine levels in muscle and caused an increase in muscle protein synthesis rates in tumor-bearing (TB)[4] and septic[5] rats. Similar to the effect of glutamine, we found that administration of an arginine-enriched total parenteral nutrition (TPN) solution also enhanced the level of glutamine in muscle and improved the muscle protein synthesis rate in Yoshida sarcoma–bearing rats.[6,7]

The pathway from arginine to glutamine consists of four steps. Arginase hydrolyzes arginine to ornithine, which is converted to glutamate semialdehyde via transamination and is then degraded to glutamate with dehydrogenase. The aims of this study were to examine the pathway from arginine to glutamine in vivo and to determine whether the presence of a tumor causes an increase in the flux of arginine to glutamine via ornithine in TB rats compared with normal rats.

The rate of incorporation of ^{14}C from U-^{14}C-arginine into glutamine was measured in control and TB rats receiving standard TPN solution. Two separate isotopic studies were performed by a constant infusion method. Glutamine flux was measured by a U-^{14}C-glutamine infusion, and the ratio of specific activity of arginine to specific activity of glutamine was determined in rats infused with U-^{14}C-arginine.

MATERIALS AND METHODS

Experimental Protocol

Thirty-six male Donryu rats (body weight, 180 to 200 g; Kuroda Animal Facility Center, Kumamoto, Japan) were maintained on a 12-hour light/dark cycle for 7 days until the start of the experimental protocol (Fig 1). On day 0, Yoshida sarcoma cells (1×10^6) were inoculated into the back of the rats (n = 18) to induce the tumor. The rats were given access to a standard rat chow and water ad libitum for 5 days. Control rats (n = 18) were housed with inoculated rats and also fed standard diet and water ad libitum. On day 5, all rats were catheterized at the jugular vein under pentobarbital anesthesia (50 mg/kg intramuscularly; Dainippon Pharmaceutical, Osaka, Japan) for TPN as described previously.[2,4] Initial TPN (day 5) was 125 kcal/kg/d total calories and 0.75 g/kg/d total nitrogen. From days 6 through 10, a full-strength diet was given to the animals (250 kcal/kg/d, 1.5 g N/kg/d). Ninety percent of the total nonprotein energy intake was given as glucose and 10% as fat to prevent essential fatty acid deficiency (Intralipid; Otsuka Pharmaceutical, Tokushima, Japan). A standard amino acid solution was administered as a nitrogen source (Moriprone

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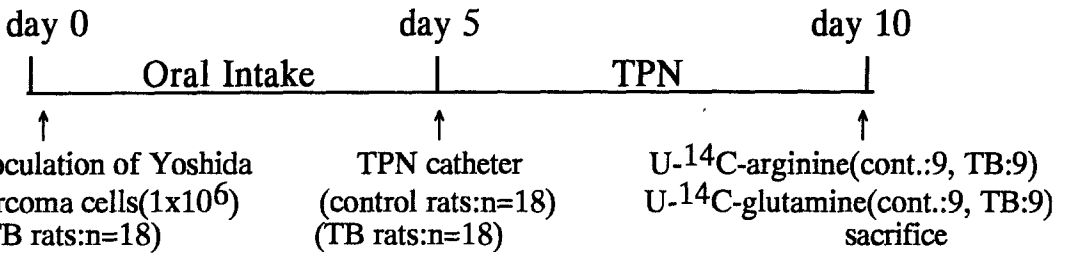

Fig 1. Experimental protocol. On day 0, Yoshida sarcoma cells were inoculated into the back of the rats (n = 18), and the rats were given access to water and rat chow ad libitum. On day 5, a TPN catheter was inserted into the jugular vein in all animals and TPN was begun. On day 10, either U-^{14}C-arginine or U-^{14}C-glutamine was infused continuously for 6 hours.

F; Morishita Pharmaceutical, Osaka, Japan). Adequate amounts of minerals, vitamins, and trace minerals were added to the infusate as described previously.[4,5] On day 10, two separate isotope infusions in the TPN solution were administered. Nine control and nine TB rats received L-[^{14}C(U)]-arginine (2 μCi/h/rat; Dupont NEN, Boston, MA) by a 6-hour constant infusion, and the remaining control and TB rats received an infusion of L-[^{14}C(U)]-glutamine (2 μCi/h/rat; Dupont NEN) for 6 hours. The animals were killed, and the blood was collected at the end of isotope infusion. The blood was centrifuged at 2,500 rpm for 15 minutes, and the plasma was stored at −70°C until assayed.

Analytical Procedures

Plasma (0.3 mL) was mixed with 2 mL 4% sulfosalicylic acid to precipitate protein. The supernatant was separated by centrifugation at 10,000 rpm for 20 minutes. The supernatant was lyophilized to dryness and then reconstituted with 1 mL 0.5N NaOH. The resulting solution (100 μL) was injected into a high-performance liquid chromatograph ([HPLC] Hitachi, Tokyo, Japan) to separate each amino acid, and fractions were collected (Toyo Advantec, Tokyo, Japan) for subsequent analysis of ^{14}C. The HPLC was equipped with a fluorescence detector (Hitachi) and a C_{18} column (Waters Japan, Osaka, Japan). Mobile phase A was composed of 50 mmol/L Na_2HPO_4 (Wako, Osaka, Japan), 50 mmol/L NaOAC (Wako), tetrahydrofuran (Wako), and methanol, which were filtered by a membrane filter (Toyo Advantec). Mobile phase B was methanol (65%) and H_2O (35%). Two gradient programs were used for separation of these amino acids. The gradient for separation of arginine and glutamine from the other amino acids was initially 80% A and 20% B. Solution A was reduced to 75% over 5 minutes, and to 70% by 15 minutes. Under these conditions, retention times for glutamine and arginine were 8.5 and 11.5 minutes, respectively. Separation of ornithine from the other amino acids required 75% A and 25% B initially. Solution A was reduced to 40% over 5 minutes, to 20% by 10 minutes, and to 0% by 15 minutes. The retention time for ornithine was 17.00 minutes. Each aliquot of glutamine, arginine, or ornithine was collected separately with an automatic fraction collector and analyzed for ^{14}C by liquid scintillation counting (Aloka LSC 563; Aloka, Tokyo, Japan).

Methods of Calculation

The rate of arginine production (micromoles per kilogram per hour) was calculated from the data obtained with U-^{14}C-arginine, using the formula, endogenous arginine production rate = $(I_{arg}/Sp_{arg}) - IN_{arg}$, where I_{arg} is the isotope infusion rate of arginine (dpm per kilogram per hour), Sp_{arg} is the specific activity of arginine in plasma at steady state (dpm per micromole arginine), and IN_{arg} represents the intake of arginine (micromoles per kilogram per hour) via TPN solution. Since five of the six carbons from U-^{14}C-arginine are incorporated into ornithine and glutamine, the ratio of carbon skeletons of ornithine derived from arginine (Orn from Arg) and the ratio of carbon skeletons of glutamine derived from arginine (Gln from Arg) were given by the following formulas: Orn from Arg (%) = $(6 \times Sp_{orn})/(5 \times Sp_{arg}) \times 100$ and Gln from Arg (%) = $(6 \times Sp_{gln})/(5 \times Sp_{arg}) \times 100$. Sp_{orn} and Sp_{gln} are the specific activity of ornithine and glutamine in plasma (dpm per micromole) derived from U-^{14}C-arginine, respectively.

The rate of glutamine production (micromoles per kilogram per hour) was calculated from the infusion of U-^{14}C-glutamine using the equation, glutamine production rate = I_{gln}/Sp_{gln}, where I_{gln} is the isotope infusion rate of glutamine (dpm per kilogram per hour) and Sp_{gln} is the specific activity of glutamine in plasma at steady state (dpm per micromole glutamine) (Fig 2). We did not take into account the intake of glutamine in this equation, because the TPN solution did not contain

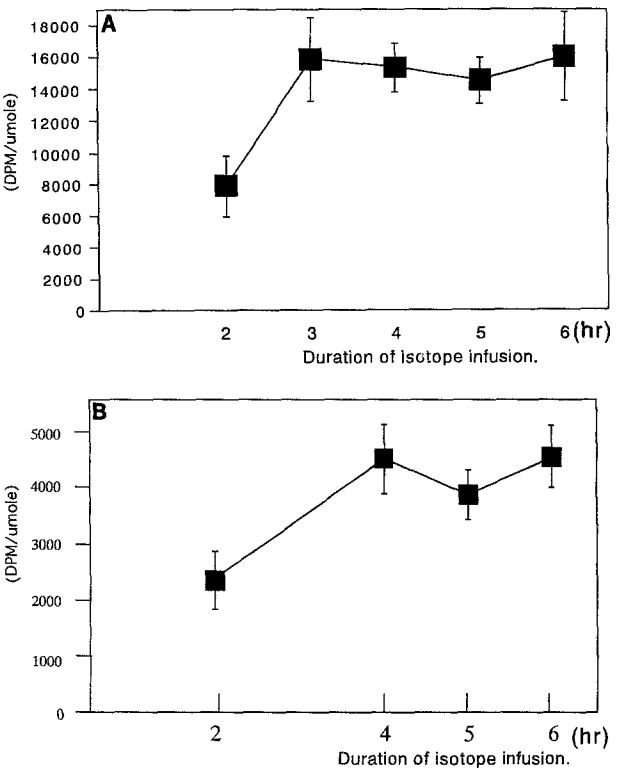

Fig 2. Verification of steady-state specific activity of arginine and glutamine in the plasma. (A) TB received U-^{14}C-arginine at a rate of 2 μCi/h/rat and were killed at 2, 3, 4, 5, and 6 hours of infusion. Data are the mean ± SEM from 3 rats at each time point. Steady-state arginine specific activity was achieved in 3 hours of isotope infusion. (B) TB received a continuous infusion of U-^{14}C-glutamine at a rate of 2 μCi/h/rat and were killed at 2, 4, 5, and 6 hours of infusion. Data are the mean ± SEM from 4 rats at each time point. A reasonable plateau was obtained in 4 hours of isotope infusion.

Table 1. Change in Body Weight (g) During 5 Days of TPN and Tumor Weight (g) on Day 10

Group	No.	BW1	BW2	BW2 − BW1	Tumor Weight
Control	18	233.2 ± 6.2	238.3 ± 5.7	5.2 ± 4.0	—
TB	18	231.5 ± 4.8	225.9 ± 5.6	−5.6 ± 2.9*	1.9 ± 0.5

NOTE. BW was measured on days 5 (BW1) and 10 (BW2). Tumor weight was measured on day 10.
Abbreviation: BW, body weight.
*$P < .05$ *v* control.

glutamine and the amount of tracer U-^{14}C-glutamine added was negligible compared with the large tissue pool.

The rate of glutamine production (micromoles per kilogram per hour) from arginine carbon skeleton (Arg to Gln) was calculated by the modified formula of Yoshida et al[2]: Arg to Gln = (glutamine production) × (Gln from Arg [%])/100.

Statistical Analysis

All data are expressed as the mean ± SEM. Statistical analysis was made by ANOVA using a Macintosh SE/30 (Apple Computers, Cupertino, CA; Statview 412 supplied by Abacus Concepts, Berkeley, CA).

Differences between means are considered significant at $P < .05$.

RESULTS

Control rats gained body weight during the 5 days of TPN, and TB rats lost weight despite the added weight of the tumor (Table 1). The presence of tumor caused a significant reduction in plasma glutamine levels (control 788.3 ± 73.2 *v* TB 565.2 ± 42.9 μmol/L, $P < .05$; Table 2). In contrast, glutamine production was significantly increased in TB rats compared with control animals (control 2,273.9 ± 192.0 *v* TB 3,809.4 ± 166.1 μmol Gln/kg/h, $P < .05$; Table 3). No difference was noted for plasma arginine levels of TB rats compared with control rats (control 189.6 ± 22.3 *v* TB 232.5 ± 25.3 μmol/L, NS; Table 2), but endogenous arginine production was greater in TB rats than in control rats (control 821.6 ± 51.3 *v* TB 1,253.3 ± 138.6 μmol Arg/kg/h, $P < .05$; Table 3). When U-^{14}C-arginine was infused in the animals, the presence of a tumor caused a significant increase in the ratio of specific activity of ornithine to specific activity of arginine (Orn from Arg, control 51.5% ± 10.9 *v* TB 77.4% ± 8.9%, $P < .05$; Table 3) and the ratio of specific activity of glutamine to specific activity of arginine in the plasma (Gln from Arg, control 1.8% ± 0.6 *v* TB 5.6% ± 1.7%, $P < .05$; Table 3). Glutamine production derived from the carbon skeleton of arginine was significantly greater in TB rats than in control rats (Arg to Gln, control 41.0 ± 11.9 *v* TB 213.3 ± 66.1 μmol/kg/h, $P < .01$; Table 3).

DISCUSSION

The continuous infusion method requires that the specific activity of infused labeled amino acid reaches a plateau in tissue amino acid pools. In rats, it takes 3 hours to equilibrate a primary pool with an isotopical infusion of amino acids.[8] Similarly, steady-state arginine specific activity was achieved with 3 hours' constant infusion at the rate of 2 μCi/h/rat (Fig 2A). Although the glutamine pool is large, a reasonable plateau was obtained with a 4-hour continuous infusion of U-^{14}C-glutamine at the rate of 2 μCi/h/rat in TB rats (Fig 2B). Indeed, the present set of values for glutamine production with a 6-hour continuous infusion are the same as in our previous report in control rats with an 8-hour infusion of 5-^{13}C-glutamine.[2]

Characteristic features of protein turnover in TB animals include increased proteolysis and decreased muscle protein synthesis.[4,9] Kawamura et al[10] reported that a loss of body weight and alterations of protein metabolism due to the fibrosarcoma were only observed after the tumor had reached considerable size (15.2 g, or ~10% of body mass). However, in the present study, 2 g of the tumor, or less than 1% of body mass, caused a loss of body weight on day 10. Similarly, Tayek et al[11] reported that the growth of nontumorous body was impaired when the tumor of Yoshida sarcoma was detectable 5 days after the inoculation. Since tumor weight in Yoshida sarcoma was approximately 30 g 18 days after inoculation and in the fibrosarcoma 15 g 16 days after inoculation, the faster growth rate in Yoshida sarcoma versus fibrosarcoma may lead to the loss of body weight in rats burdened with a small Yoshida sarcoma.[10,11] In addition, since the fractional synthesis rate was faster in the small tumor than in the large tumor,[12] the plasma glutamine level was decreased due to a utilization of glutamine in the small but rapidly growing tumor.[13]

Although arginine levels in plasma were unaltered, arginine production was increased in TB rats in the present study. One reason for the unchanged plasma arginine levels was probably that arginine was provided in the TPN solution. Arginine production reflects protein breakdown and de novo arginine synthesis in liver and kidney.[14] Yu et al[15] reported that endogenous arginine production derived from protein breakdown was

Table 2. Plasma Levels of Glutamine, Arginine, and Ornithine

Group	Gln	Arg	Orn
Control (μmol/L)	788.3 ± 73.2	189.4 ± 25.3	122 ± 32
TB (μmol/L)	565.2 ± 42.9*	232.5 ± 22.0	124 ± 40

NOTE. Levels were determined after infusion of isotope dissolved in TPN.
*$P < .05$ *v* control.

Table 3. Characteristics of Glutamine, Ornithine, and Arginine Metabolism Determined From Steady-State Isotope Infusion

Parameter	Control	TB
Gln production (μmol/kg/h)	2,273.9 ± 192.0	3,809.5 ± 166.1*
Arg production (μmol/kg/h)	821.6 ± 51.3	1,253.3 ± 138.6*
Orn from Arg (%)	51.5 ± 10.9	77.4 ± 8.9*
Gln from Arg (%)	1.8 ± 0.6	5.6 ± 1.7*
Arg to Gln (μmol/kg/h)	41.0 ± 11.9	213.3 ± 66.1†

Abbreviations: Orn from Arg, ratio of specific activity of ornithine to specific activity of arginine in rats receiving U-^{14}C-arginine; Gln from Arg, ratio of specific activity of glutamine to specific activity of arginine in rats receiving U-^{14}C-arginine; Arg to Gln, glutamine production derived from arginine.
*$P < .05$, †$P < .01$: *v* control.

increased in burn patients compared with healthy adults, although arginine de novo synthesis was unaltered. In the present study, the increase in arginine production was probably due to an enhancement of protein breakdown induced by the presence of tumor.[4]

In the present study, glutamine synthesis derived from arginine via ornithine was fivefold higher in TB rats than in controls. There are three possible sites of glutamine synthesis derived from arginine. The liver and brain convert arginine to glutamate, and the activity of glutamine synthetase is high in these tissues.[16,17] However, in the liver, enzymes of the urea cycle are present in periportal hepatocytes, whereas glutamine synthetase is found only in perivenous hepatocytes.[18] Furthermore, Castillo et al[19] proposed that plasma and hepatic urea arginine pools are distinct, suggesting that it is unlikely that glutamine synthesis was derived from arginine in the liver. Although arginine is converted to glutamate semialdehyde via ornithine in muscle,[20] there is no evidence that glutamate semialdehyde dehydrogenase is present in muscle. However, since muscle is the main site of glutamine synthesis due to its high glutamine synthetase activity,[21] it is not implausible that arginine is a direct precursor of glutamine in muscle.

Although a solution enriched with arginine and BCAA has been shown to enhance glutamine levels in skeletal muscle,[6,7] the present study showed that only 6% of glutamine was derived from arginine in TB rats. We previously reported that only 3% of glutamine production was converted from leucine in septic rats; nevertheless, this value was higher than in control rats.[2] Thus, the small increase in leucine and arginine conversion to glutamine cannot explain either a large increase in glutamine production in TB rats or an elevation in glutamine levels in skeletal muscle with infusion of BCAA and arginine–enriched solution in TB rats as compared with standard TPN solution.

In summary, the pathway of arginine to glutamine via ornithine appears to function in vivo. Glutamine synthesis derived from the carbon skeleton of arginine via ornithine was increased in TB rats. However, since the fraction of glutamine production derived from arginine was only 6% in TB rats, the effect of the BCAA and arginine–enriched solution on the increased glutamine level in skeletal muscle was probably the result of an inhibition of glutamine release from skeletal muscle with administration of the solution.

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