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• BASIC RESEARCH •

Influence of methionine/valine-depleted enteral nutrition on nucleic acid and protein metabolism in tumor-bearing rats

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Abstract

AIM: To investigate the effects of methionine/valine-depleted enteral nutrition (EN) on RNA, DNA and protein metabolism in tumor-bearing (TB) rats.

METHODS: Sprague-Dawlley (SD) rats underwent jejunostomy for nutritional support. A suspension of Walker-256 carcinosarcoma cells was subcutaneously inoculated. 48 TB rats were randomly divided in 4 groups: A, B, C and D. The TB rats had respectively received jejunal feedings supplemented with balanced amino acids, methionine-depleted, balanced amino acids and valine-depleted for 6 days before injection of 740 KBq ^{3}H- methionine/valine via jejunum. The ^{3}H incorporation rate of the radioactivity into RNA, DNA and proteins in tumor tissues at 0.5, 1, 2, 4 h postinjection of tracers was assessed with liquid scintillation counter.

RESULTS: Incorporation of ^{3}H into proteins in groups B and D was (0.500±0.020) % to (3.670±0.110) % and (0.708±0.019) % to (3.813±0.076) % respectively, lower than in groups A [(0.659±0.055) % to (4.492±0.108) %] and C [(0.805±0.098) % to (4.180±0.018) %]. Incorporation of ^{3}H into RNA, DNA in group B was (0.237±0.075) % and (0.231±0.052) % respectively, lower than in group A (P<0.01). There was no significant difference in uptake of ^{3}H by RNA and DNA between group C and D (P>0.05).

CONCLUSION: Protein synthesis was inhibited by methionine/valine starvation in TB rats and nucleic acid synthesis was reduced after methionine depletion, thus resulting in suppression of tumor growth.

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INTRODUCTION

Parenteral nutrition (PN) is now a supportive therapy commonly used for cancer patients. However, some studies have suggested that PN with amino acid balanced solutions may prompt tumor growth[1-3]. Previous studies have shown that tumor growth was inhibited by a diet or PN lacking in methionine/valine. However, the mechanism is not yet known[4-15]. In this study, we prepared methionine/valine-free amino acid imbalance solutions to investigate the effects of methionine/valine depleted EN on RNA, DNA and protein metabolism in TB rats.

MATERIALS AND METHODS

Radiopharmaceuticals

^{3}H-methionine (^{3}H-Met, specfic activity of 148 MBq· mg^{-1}) and ^{3}H-valine (^{3}H-Val, specfic activity of 240 MBq· mg^{-1}) was purchased from Chinese institute of atomic energy. The radiochemical purity was over 95 %.

Catheterization of jejunostomy

SD rats weighing (160±20) g were purchased from the animal center of Wuhan University, China. They were allowed to acclimate for one week. After fasting for 12 hours, rats were anesthetized with i.p. sodium pentobarbital (40 mg· kg^{-1}). The animals were undergone catheterization of jejunostomy (day 0). A silicone rubber catheter (2 mm ID, 3 mm OD) was inserted into the proximal jejunum. The catheter passed through a subcutaneous tunnel and emerged between the scapulae. The catheter was sutured to the animal' s back to protect the lines and was connected to a swivel so that animals can move without any restrictions in individual metabolic cages. The cannulation system consists of an microinfusion pump, a swivel, rat-harness and a silicone-tube-jejunostomy. Coprophagy was prevented by an own model of faecal collection cup. Animals were fasted for 48 hours after operation but they were provided with water ad libitum, and then given normal rat diets.

Preparation of TB rats

Walker-256 carcinosarcoma cells were obtained from Chinese Center of Culture Preservation. On day 0, the rats were inoculated subcutaneously in the right flank with 10^7 tumor cells of approximately 0.1 ml of cell suspension. Tumors were palpable in 7 days after transplantation.

Jejunal feeding

Enteral feedings were found to be a safe and cost-effective method for providing nutrition to cancer-bearing patients. On day 8, 48 TB rats were randomly divided into four groups (12 rats per group) and received enteral nutrition (jejunal feeding): Group A: TB rats were fed enteral nutrition solutions composed of balanced amino acids for 6 days before injection of 740 KBq ^{3}H-MET.

Group B: TB rats were fed methionine-depleted enteral nutrition solutions for 6 days before injection of 740 KBq ^{3}H-Met.

Group C: TB rats were fed enteral nutrition solutions composed of balanced amino acids for 6 days before injection of 740 KBq ^{3}H-Val.

Group D: TB rats were fed valine-depleted enteral nutrition solutions for 6 days before injection of 740 KBq ^{3}H-Val.

TB rats received continuous jejunal tube infusion with pump

for nutritional support at a daily dose of 330 ml· kg^{-1}, non-protein calorie was approximately 1160K J· kg^{-1}. A microinfusion pump was used for constant administration of EN solutions. TB rats were not fed during the entire infusion experiment, however they had free access to water.

Composition of amino acid solutions

Table 1 lists the components of amino acid solutions.

Table 1 Composition of amino acid solutions ($g \cdot L^{-1}$)

Amino acids	Balanced amino acids (Group A, C)	Methionine-depleted (Group B)	Valine-depleted (Group D)
Isoleucine	5.5	5.5	5.5
Leucine	7.5	7.5	7.5
Lysine	7.0	7.0	7.0
Methionine	6.0	-	6.0
Phenylalanine	4.0	4.0	4.0
Threonine	5.0	5.0	5.0
Tryptophan	1.5	1.5	1.5
Valine	6.0	6.0	-
Arginine	6.0	6.0	6.0
Histidine	3.0	3.0	3.0
Proline	4.0	4.0	4.0
Tyrosine	1.0	1.0	1.0
Alanine	20.0	20.0	20.0
Glycine	7.5	7.5	7.5
Aspartic acid	4.0	4.0	4.0
Total amino acid	88.0	82.0	82.0
Total N	14.1	13.1	13.1

Composition of EN solutions

Table 2 summarizes the daily EN compositions infused into various groups.

Table 2 Compositions of EN solutions ($ml \cdot L^{-1}$)

Amino acids	Balanced amino acids (group A, C)	Methionine-depleted (group B)	Valine-depleted (group D)
Amino acid solutions	350	350	350
50 % Glucose	300	300	300
20 % Intralipid	100	100	100
Electrolytes, vitamine	250	250	250
Total calorie ($KJ \cdot L^{-1}$)	3 513.3	3 507.9	3 507.9
Total N ($g \cdot L^{-1}$)	4.9	4.6	4.6
Non-protein calorie / N	122	131	131

Specimen sampling

After the infusions were completed, three rats per group were respectively killed by cervical dislocation at 0.5, 1, 2 and 4 hours postinjection of tracers. The whole tumor was dissected and used for the tissue uptake of radioactivity.

Nucleic acid and protein analysis

To assess the incorporation of the radioactivity into macromolecular materials, portions of the tumor tissues (70-120 mg) were divided into the acid-soluble fraction (ASF) and the acid-precipitate fraction (APF). Radiolabeled APF was divided into four fractions: lipids, RNA, DNA and proteins. To analyze ^{3}H-Met and ^{3}H-Val metabolites, the tumor tissues were homogenated in 1 ml of ice-cold 0.4 M $HClO_4$. The homogenate was centrifuged at 3 000 rpm for 5 min. The precipitate was resuspended in 1 ml of 0.4 M $HClO_4$. This wash was repeated twice. The precipitate was resuspended in 5 ml of $CHCl_3$: CH_3OH (2:1,V/V). After centrifugation at 3 000 rpm for 10 min, the $CHCl_3$: CH_3OH phase was separated. This extraction was repeated twice. The combined $CHCl_3$: CH_3OH fraction contains radiolabeled lipids. The precipitate was dissolved in 1 ml of 0.3 M KOH. After incubation of the solution at 37 ℃ for 1 hour to hydrolyze RNA, 0.32 ml of 3 N $HClO_4$ was added. The mixture was kept on ice for 5 min. The precipitate was then separated and washed with 1 ml 0.5 M $HClO_4$ as described above. The combined supernatant was designated as the alkaline-labile fraction containing the RNA hydrolysate. The precipitate was resuspended in 1 ml of 0.5 M $HClO_4$ and heated at 90 ℃ for 15 min to hydrolyze DNA. The solution was kept on ice for 5 min, and precipitate was separated and washed with 0.4 M $HClO_4$ twice. The combined supernatant and the final precipitate were assessed as the acid-labile fraction containing hydrolysates of DNA and protein fraction, respectively.

The radioactivities of fractions were counted by liquid scintillation counter. The tissue radioactivity was expressed as differential uptake ratio (DUR).

$$DUR = \frac{\text{Counts of tumor tissue (cpm)/sample weight (g)}}{\text{Injection dose counts (cpm)/body weight (g)}}$$

Statistical analysis

Student *t* test was used to examine the data. The difference was considered significant when *P* value was less than 0.05.

RESULTS

Three TB rats died of intestinal fistula, diarrhea, infection of abdominal cavity. Table 3 represented incorporation of ^{3}H into nucleic acids and proteins in TB rats after treatment.

Table 3 Incorporation (DUR,%) of ^{3}H into nucleic acids and proteins in TB rats after treatment

Group		0.5 h	1 h	2 h	4 h
RNA	A	0.208±0.002	0.300±0.002	0.349±0.007	0.405±0.007[c]
	B	0.149±0.012	0.249±0.009	0.260±0.010	0.389±0.010
	C	0.200±0.007	0.250±0.036[b]	0.283±0.029[ac]	0.326±0.014[c]
	D	0.180±0.013	0.210±0.024	0.300±0.034	0.320±0.030[b]
DNA	A	0.210±0.013	0.300±0.020	0.339±0.039	0.400±0.002[c]
	B	0.179±0.010[a]	0.204±0.039[a]	0.240±0.028[a]	0.300±0.015[b]
	C	0.200±0.011	0.250±0.040	0.283±0.031[c]	0.340±0.057[c]
	D	0.180±0.015	0.220±0.024	0.308±0.007	0.320±0.035
Proteins	A	0.659±0.055	2.410±0.149	3.450±0.125	4.492±0.108[c]
	B	0.500±0.020[b]	2.000±0.203[b]	2.890±0.090[bc]	3.670±0.110[b]
	C	0.805±0.098	2.510±0.010	3.540±0.101[c]	4.180±0.018[c]
	D	0.708±0.019	1.887±0.020[b]	2.916±0.085[b]	3.813±0.076[b]

[a]$P<0.05$, [b]$P<0.01$, *vs* group A or C. [c]Number of rats=2.

DISCUSSION

Influence of Methionine/Valine-depleted enteral nutrition on protein metabolism in TB rats

Patients with malignant tumors often show severe protein-amino acid metabolism disorder and uncorrectable negative nitrogen balance. Researchers have begun to reconsider the prescription of amino acid imbalance solution for cancer patients. Total parenteral nutrition deprived of methionine or valine cause tumor growth inhibition, but also have no significantly negative influences on the host animals[16-18].

Table 3 shows the ^{3}H incorporation rate in tumor tissues at

various times after ^{3}H-Met/Val injections. Regardless of Methionine/Valine-depleted enteral nutrition, the radioactivity into nucleic acids and proteins increased with time. In proteins we found an accumulation of the label which was up to 3-10-fold higher than in DNA and RNA. It represents the principle pathway for methionine and valine anabolism. Accumulation of ^{3}H-Met/Val into malignant tissue is thought to be due to amino acid metabolism of cancer cells such as increased active transport and incorporation of amino acid into protein fractions. In the complete absence of Methionine or Valine, the ^{3}H incorporation rate of the radioactivity into proteins in tumor tissues was from 75.8 % to 87.9 % of the control value. That is to say, in agreement with Xiao' s study[5], protein synthesis was inhibited by methionine/valine depletion, in this case suppressing tumor growth[19-26].

Although essential amino acids are indispensable for physical well-being, the body lacks the ability to synthesize these compounds. Amino acids are an important materials of protein synthesis, amino acid imbalance are considered to principally involving alterations in intracellular protein synthesis, the deprivation of essential amino acids (Met, Val) leads to inhibit activity of tumor growth[27,28].

Influence of Methionine/Valine-depleted enteral nutrition on RNA and DNA in TB rats

Methionine adenosyltransferase is the enzyme which is responsible for the synthesis of S-adnosyl-L-methionine (SAM) using methionine and adnosine triphosphate (ATP). Most of SAM are used in transmethylation reaction in which methyl groups are added to compounds and SAM is converted to S-adenosylhomocysteine. SAM is the principal biological methyl donor. SAM can easily transfer its methyl group to a large variety of acceptor substrates including rRNA, tRNA, mRNA, DNA, proteins, phospholipides, biological amines, and a long list of small molecules[29-33]. So ^{3}H-Met is also incorporated into nucleic acids by transmethylation via S-adenosyl-L-methionine. Methionine depleted enteral nutrition can decrease methylation of tumor tissues and lead to further reduction in nucleic acid synthesis and inhibition of cancer growth at molecular levels.

Table 3 showed that the RNA and DNA incorporation rate in group B was lower than in control group (group A). Based on these findings, cancer cells were known to have lower levels of DNA and RNA synthesis on methionine-depleted enteral nutrition.

Theoretically, it is considered that ^{3}H-Val is incorporated into proteins but not into other high-molecular materials such as nucleic acids. The incorporation of ^{3}H-Val was detected in nucleic acids at negligible amounts, which possibly reflects contamination by labeled proteins during the experimental processes. However, because no metabolic pathway for the DNA incorporation of ^{3}H-Val is considered, the radioactivity in the acid-labile fraction is probably derived from basic proteins such as chromosomal histones.

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