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• BASIC RESEARCH •

Effect of complex amino acid imbalance on growth of tumor in tumor-bearing rats

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Abstract

AIM: To investigate the effect of complex amino acid imbalance on the growth of tumor 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. TB rats were randomly divided into groups A, B, C and D according to the formula of amino acids in enteral nutritional solutions, respectively. TB rats received jejunal feedings supplemented with balanced amino acids (group A), methionine-depleted amino acids (group B), valine-depleted amino acids (group C) and methionine- and valine-depleted complex amino acid imbalance (group D) for 10 days. Tumor volume, inhibitory rates of tumor, cell cycle and life span of TB rats were investigated.

RESULTS: The G_0/G_1 ratio of tumor cells in group D (80.5±9.0) % was higher than that in groups A, B and C which was 67.0±5.1 %, 78.9±8.5 %, 69.2±6.2 %, respectively ($P<0.05$). The ratio of S/G_2M and PI in group D were lower than those in groups A, B and C. The inhibitory rate of tumor in groups B, C and D was 37.2 %, 33.3 % and 43.9 %, respectively ($P<0.05$). The life span of TB rats in group D was significantly longer than that in groups B, C, and A.

CONCLUSION: Methionine/valine-depleted amino acid imbalance can inhibit tumor growth. Complex amino acids of methionine and valine depleted imbalance have stronger inhibitory effects on tumor growth.

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INTRODUCTION

Malnutrition is encountered everyday in cancer patients and is associated with severe protein-amino acid metabolic disorder, uncorrectable negative nitrogen balance and low immune function[1-6]. Enteral nutrition (EN) and parenteral nutrition (PN) are both safe and effective methods of administering nutrients in cancer patients[7-9]. But PN with amino acid balanced solutions may prompt tumor growth[10-12]. Based on Harper's concept of amino acid imbalance, EN/TPN preparations with depleted or enriched specific amino acids produce tumor growth inhibition[13-18]. Previously, we found methionine/valine-depleted (0), low tyrosine (0.5 g/L) and arginine-enriched (6 g/L) complex amino acid imbalance solutions were the most rational formula in tumor-bearing (TB) rats[19]. In this study, we aimed to investigate the effect of complex amino acid imbalance on the growth of tumor.

MATERIALS AND METHODS

Animals

SD rats weighing 170±20 g were purchased from the Experimental Animal Center of Wuhan University (Wuhan, China) and fed with a stock rat diet *ad libitum*. The animals were maintained on a 12-hour light/12-hour dark cycle at ambient temperature of (23±2) ℃ and housed for 7 days before the experiment.

Catheterization of jejunostomy

After fasted for 12 hours, the rats (n=60) were anesthetized by intraperitoneal administration of 40 mg· kg^{-1} pentobarbital. They were then undergone catheterization during jejunostomy (day 0). A silicone rubber catheter with an internal diameter of 2 mm and an external diameter of 3 mm was inserted into the proximal jejunum. The catheter passed through a subcutaneous tunnel and emerged between the scapulae. The catheter was then mounted on a harness, passed through a protective coil, and connected to a swivel so that the animals could move without any restrictions in individual metabolic cages. The cannulation system consisted of a microinfusion pump, a swivel, rat-harness and a silicone-tube-jejunostomy. The rats were fasted for 48 hours after operation but were provided with water *ad libitum*, and then given normal rat diet.

Preparation of TB rats

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

Tumor weights and inhibitory rates of tumor

Tumors were palpable 7 days after transplantation. Measurements were made at the tumor site. The lengths of the major, minor axes and depth were measured with calipers. Growth of the tumor was evaluated every 3 days. Tumor volumes during experiments were calculated according to the following equation: $V=LWDp/6$. Where V is the tumor volume (mm^3), L is the length, W is the width and D is the depth of a solid tumor (mm). Inhibitory rates of tumor = (tumor volume of control group- tumor volume of experimental group)/tumor volume of control group×100 %.

Experimental groups and jejunal feeding

On day 8, 48 TB rats were randomly divided into four groups

(12 rats per group) according to the solutions administered: an amino acid balance solution (group A), methionine-depleted amino acid solution (group B), valine-depleted amino acid solution (group C), and methionine and valine-depleted complex amino acid solution (group D). They were administered enteral nutritional solutions (jejunal feeding) for 10 days. During EN, the rats were individually housed in metabolic cages. The compositions of EN solution infused to each rat are summarized in Table 1.

Administration methods

TB rats received continuous jejunal tube infusion for nutritional support at a daily dose of 330 ml· kg^{-1}, by means of a microinfusion pump (Sino-Swed Pharmaceutical Corp. Ltd. China). Non-protein calorie per day was approximately 1 104 K J· kg^{-1}. TB rats were not fed during the entire infusion experiment, however, they had free access to water.

Compositions of amino acid solutions

Table 1 lists the components of amino acid solution in four groups.

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

Amino acids	Group A	Group B	Group C	Group D
Isoleucine	5.5	5.5	5.5	5.5
Leucine	7.5	7.5	7.5	7.5
Lysine	7.0	7.0	7.0	7.0
Methionine	6.0	–	6.0	–
Phenylalanine	4.0	4.0	4.0	4.0
Threonine	5.0	5.0	5.0	5.0
Tryptophan	1.5	1.5	1.5	1.5
Valine	6.0	6.0	–	–
Arginine	6.0	6.0	6.0	6.0
Histidine	3.0	3.0	3.0	3.0
Proline	4.0	4.0	4.0	4.0
Tyrosine	1.0	1.0	1.0	1.0
Alanine	20.0	20.0	20.0	20.0
Glycine	7.5	7.5	7.5	7.5
Aspartic acid	4.0	4.0	4.0	4.0
Total amino acid	88.0	82.0	82.0	76.0
Total nitrogen	14.1	13.1	13.1	12.2

Compositions of EN solution

1 000 ml EN solution was composed of 350 ml of amino acid preparation (Table 1) supplemented with 300 ml of 50 % glucose, 100 ml of 20 % Intralipid (Sino-Swed Pharmaceutical Corp. Ltd. China), 20 ml of Soluvit, 20 ml of Vitalipid, 20 ml of Addamel and 190 ml of 0.9 % saline.

Specimen sampling

At the end of an administration period, 6 rats per group were respectively killed by cervical dislocation. The whole tumor was dissected and examined.

Cell cycle position measurement

Sections about 50 μm thick were cut from tumor tissues and washed 3 times in phosphate-buffered saline. Cell kinetics were measured by flow cytometry (FCM, PASIII, Partec Company, Germany).

Life span of TB rats

The remaining 6 rats per group were given solid food (Experimental Animal Center of Wuhan University, Wuhan, China) and water *ad libitum* according to their body weight until they died of advanced cancer. The life span of each rat in terms of median survival time (MST) was observed.

Statistical analysis

All results were presented as mean ±SD. Comparisons of the four groups were made using Uni-variate ANOVA test. The difference was considered significant when *P* value was less than 0.05.

RESULTS

Animals

Two rats in groups A, C and two rats in group D died of intestinal fistula, diarrhea, infection of abdominal cavity during enteral nutrition, respectively.

Changes in tumor cell cycle

Tumor-selective cell cycle arrest occurred in the S-G_2 phase during methionine depleted enteral nutrition. The distribution of cancer cell cycle was not obviously affected during valine starvation. The percentages of tumor cells in G_0G_1 phase in groups B and D were significantly higher than that in group A while the percentages of S phase cells in groups B and D were obviously lower than that in group A ($P<0.05$). There was no statistical difference between the percentages of G_2M cells in groups B and D and that in group A ($P>0.05$, Table 2).

Table 2 Distribution of cancer cell cycle after EN treatment (%)

Phase	Group A	Group B	Group C	Group D
G_0G_1	67.0±5.1	78.9±8.5[ac]	69.2±6.2[b]	80.5±9.0[ac]
S	20.1±1.8	11.8±2.9[ac]	19.9±3.0[b]	10.2±2.1[ac]
G_2+M	12.9±3.2	9.2±3.1	10.9±2.5	9.4±3.8
PI(S+G_2+M)	33.0±4.3	21.0±5.0[ac]	30.8±5.6[b]	20. 5±2.8[ac]

[a]$P<0.05$, *vs* group A, [b]$P<0.05$, *vs* group B, [c]$P<0.05$, *vs* group C.

Tumor volumes and inhibitory rates of tumor

Tumor volume had no statistical difference among each group before treatment. On day 10 of enteral nutrition, tumor growth in amino acid imbalance groups (groups B, C and D) was significantly lower than that in control group. The most remarkable inhibitory effect on tumor growth was found in complex amino acid imbalance group (group D) ($P<0.05$). The inhibitory rate of tumor (IRT) in groups B, C and D was respectively 37.2 %, 33.3 % and 43.9 % (Table 3).

Table 3 Changes in tumor volumes, IRT and MST before and after treatment

Group	Tumor volumes		IRT(%)	MST(d)
	Before	After		
Group A	0.028±0.015	2.85±0.43	...	26.8±1.5
Group B	0.039±0.010	1.79±0.56[a]	37.2	32.0±2.6[a]
Group C	0.033±0.020	1.90±0.30[ab]	33.3	35.6±3.2[a]
Group D	0.031±0.011	1.60±0.40[abc]	43.9	39.4±3.0[abc]

[a]$P<0.05$, *vs* group A, [b]$P<0.05$, *vs* group B, [c]$P<0.05$, *vs* group C.

Life span of TB rats

The median survival time (MST) in complex amino acid imbalance group was (39.4±3.0) days, as compared with (32.0±2.6) days in methionine-depleted group and (35.6±3.2) days in valine-depleted group (Table 3).

DISCUSSION

Influence of balanced amino acids on tumor growth

Compared with normal cells, the metabolism of tumor cells is significantly accelerated. *In vivo*, cancer cells have been known to have higher levels of protein synthesis, accompanied by a more active uptake of glucose and amino acids (nitrogen trap), and undergo more rapid differentiation and proliferation than healthy cells[20]. Amino acids are important materials of protein synthesis, Supplement of balanced amino acids results in the greatest increase of tumor cell cycles, thus tumor tissues compete with host tissues for nitrogen substrates. Nucleic acid and protein synthesis are increased, and tumor growth is accelerated. Table 3 indicates the experimental results of the anticancer effects of various amino acid imbalance solutions. As clearly shown in this table, tumor volume was especially large, and the median survival time was short after TB rats were administrated with balanced amino acid solutions (group A).

Influence of methionine/valine-depleted amino acid imbalance on tumor growth

Methionine dependency of many malignant tumor cells has been demonstrated in previous studies. That is to say, these cells were arrested in late S/G_2 phase in methionine free cell culture media, and tumor cellular proliferation was inhibited, and normal cells were methionine independent after methionine was replaced by hemocysteine[21-26]. Methionine is the principal biological methyl donor via S-adenosyl-L-methionine (SAM). 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. Methionine dependency might be due to overutilization of methionine for transmethylation reactions resulting in a low free methionine pool and a low S-adenosylmethionie/S-adenosylhomocysteine ratio[27-31]. This directly inhibits the activity of transmethylase, thereby methionine depleted enteral nutrition can decrease methylation reaction of tumor tissues and lead to further reduction in nucleic acid synthesis and inhibition of cancer growth at molecular levels.

Our study demonstrated that tumor growth in group B was significantly slower than that in control group, the liver and peritoneum metastasis of cancer was much less in group B. It suggested that the invasive ability for metastasis be suppressed during methionine starvation. Breillout *et al* considered that methionine depletion disturbed the membrane lipids of tumor cells and inhibited their metastatic ability[32].

It was also found that valine depleted imbalance solution (group C) had a great inhibitory effect on Walker-256 carcinosarcoma cells. One possible mechanism was the alterations of intracellular protein synthesis due to deprivation of essential amino acids (Valine)[33-35]. Another possible mechanism seemed to be the inhibitory effect on the production of prolactin, which was likely to participate in tumor growth[20].

Influence of complex amino acid imbalance on tumor growth

As shown in Table 3, the most remarkable inhibitory effect on cancer growth was seen in the methionine/valine depleted complex amino acid imbalance group, followed by the methionine depleted imbalance group and then the valine depleted group. It suggested that complex amino acid imbalance solutions had the most strong anticancer effects. However, are we able to prevent the development of side effects of complex imbalance due to starvation of essential amino acids? This still needs further studies.

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