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• COLORECTAL CANCER •

Amino acid uptake in arterio-venous serum of normal and cancerous colon tissues

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Abstract

AIM: To investigate the difference of amino acid uptake between normal and cancerous colon tissues.

METHODS: Sixteen patients with colon cancer were enrolled in our study. Blood samples were taken during operations, serum amino acid concentrations of blood from cancerous or normal colon were analyzed. Amino acid uptake rate was calculated by the A-V difference and evaluated statistically.

RESULTS: Except for methionine, the uptake rate of amino acids in cancer was higher than that in normal colon (25.01% *vs* -2.29%, $P<0.01$). The amino acid uptake rate did not correlate to the size of tumor mass ($P>0.05$). There was no statistical significance in the amino acid uptake rate according to the Dukes stage, though it was higher in patients with Dukes stage C or D than that with Dukes stage B ($P>0.05$).

CONCLUSION: Abnormal synthetic metabolism of colon cancer may contribute to its higher amino acid uptake rate than that of normal colon.

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INTRODUCTION

Studies note the significance of amino acid metabolism in neoplasms, though there are lots of questions that remain to be answered[1-5]. It is helpful to improve the life quality if we take patients' nutrition into account according to their characteristic amino acid metabolism before we treat cancer patients. Studies[6,7,8,9] demonstrated that the amino acid concentrations in serum, especially essential amino acids (EAAs) but not leucine, were decreased in colon cancer patients with weight loss, but not in patients without weight changes, of which the non-essential amino acids (NEAAs), like asparagic acid, glutamine, glycine, alanine, taurine and ornithine, were slightly elevated in serum. Zheng[10] studied the changes of free amino acids in colon cancer tissue and revealed that the serum concentration of most EAAs (leucine, isoleucine, phenylalanine, threonine, lysine) and some NEAAs (tyrosine, proline, glutamine) in cancer tissue were obviously higher than those in normal tissue ($P<0.01$), while the serum concentration of most NEAAs and some EAAs (methionine, valine, histamine) was slightly elevated. There was no statistical significance. The ammonia serum concentration in cancer tissue decreased significantly ($P<0.05$). To make further investigations of the amino acid metabolism in colon cancer, we analyzed the arteral and venous (A-V) serum free amino acid uptake rates in tumor region, and tried to find the difference of amino acid metabolism between normal and cancerous colon tissue.

MATERIALS AND METHODS

Clinical materials

Sixteen patients were enrolled in this study from Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University Medical College. Patients with a median age of 57 years (range from 37 to 77) were diagnosed having colon cancer pathologically. Nine (66.7%) patients were males and in 9 patients, the mass was larger than 5 cm in size. According to Dukes stage, 10 patients were in stage B, 5 in stage C, and 1 in stage D. Before operation, hormone, blood, albumin and amino acid intake were prohibited. Five-day doses of sulfaguanidine and metronidazol were given for bowel preparations.

Sample collection and processing

A 3 mL arterial blood as sample A was collected before making division of the mesentery vessels during laparotomy, and 3 mL reflux venous blood from the tumor region was collected as sample C, another 3 mL mesentery venous blood in normal colon tissue, which was about 7 cm from the tumor margin, was collected as sample E. After incubation at 37 ℃ for an hour, the serum was collected by centrifugation, and 1mL serum mixed with 50 mg sulfosalicylic acid was placed for an hour at 4 ℃, then centrifuged at 16 000 *g* for 6 min. The supernatant was diluted with 0.02 mol/L hydrochloric acid before analysis. Each sample was analyzed twice with automatic amion-acid analyzer (Japan 835-50) 18 standard amino acids including ammonia were placed as control.

Statistical analysis

The A-V amino acid uptake rate in colon cancer (R1) was calculated by the following formula (R1=(A-C)/A×100%) and that in normal tissue (R2) was calculated as the following: R2= (A-E)/A×100% [A, C and E refer to the amino acid concentrations of samples A, C and E]. The rate was compared by means of *t* test, statistical significance was defined as $P<0.05$.

RESULTS

We measured 16 patients with 48 samples. The uptake rates of amino acids in normal and cancerous colon tissue were calculated. As is shown in Table 1, except for methionine, the uptake rates of all amino acids in cancer were higher than those

in normal colon (25.01% *vs* -2.29%, $P<0.01$). The EAAs uptake rates in cancer tissue were between 25.44% and 39.75% with a mean of 31.7% and between -8.01% and 13.5% in normal colon tissue ($P<0.01$). Methionine was 11.55% in cancer tissue but -0.13% in normal tissue. The ketogenic or ketogenic and glucogenic amino acid uptake rates had a mean of 30.86%, which was higher than that of glucogenic acids (18.7%). Sulfur-bearing amino acid uptake rates in colon cancer tissue, which were comparatively lower than the other amino acids, were higher than those in normal tissue, though there was no statistical significance. The uptake rates of some amino acids (lysine, arginine, proline, glutamine, glycine, alanine, cystine, methionine and ammonia) were negative in normal tissue, and the serum concentration of asparagic acid could not be detected in more than 10 patients.

Table 1 A-V amino acid uptake rates in normal and cancerous colon tissue

Amino acid	Sample number	R2 (%)	R1 (%)	*P* value
Threonine [1,3]	16	13.50±3.76	39.25±7.59	<0.01
Phenylalanine [1,3]	16	6.37±1.77	26.26±4.31	<0.01
Leucine [1,4]	16	8.21±2.13	36.55±6.11	<0.01
Isoleucine [1,3]	15	11.39±3.55	30.63±5.50	<0.01
Methionine [1]	16	-0.13±1.20	11.55±5.22	>0.05
Valine [1]	16	7.67±2.42	33.46±5.56	<0.01
Lysine [1,4]	16	-8.01±1.85	25.44±4.41	<0.01
Histidine [1]	16	1.06±0.94	29.83±6.37	<0.01
Proline [2]	11	-26.86±8.36	6.49±1.69	<0.02
Glutamine [2]	16	-12.42±3.12	18.70±5.23	<0.01
Ammonia [2]	16	-23.61±4.58	4.70±1.97	<0.05
Alanine [2]	16	-9.65±2.47	18.16±2.29	<0.01
Glycine [2]	16	-1.50±0.98	29.18±4.41	<0.01
Cystine [2]	13	-10.01±3.37	8.89±1.58	<0.01
Arginine [2]	16	-1.74±0.88	29.02±4.93	<0.01
Serine [2]	16	2.39±1.02	37.26±5.67	<0.01
Tyrosine [2,3]	16	6.57±1.45	26.33±3.39	<0.01

[1]Refers to essential amino acids, [2]refers to non-essential amino acids, [3]refers to ketogenic and glucogenic amino acids, [4]refers to ketogenic amino acids.

Table 2 Relationship between A-V amino acid uptake rates and tumor size in colon cancer

Amino acid	Tumor size<5.0 cm		Tumor size>5.0 cm		*P* value
	Sample number	R1 (%)	Sample number	R1 (%)	
Threonine	7	35.25±10.13	9	42.37±7.81	0.580
Phenylalanine	7	25.44±10.63	9	26.89±3.81	0.889
Leucine	7	33.02±12.85	9	39.30±6.53	0.648
Isoleucine	6	30.09±16.48	9	30.99±4.34	0.950
Methionine	7	13.53±7.47	9	9.99±8.79	0.772
Valine	7	27.63±8.37	9	37.99±8.50	0.408
Lysine	7	21.17±14.07	9	28.76±7.36	0.618
Histidine	7	25.41±10.03	9	33.26±7.26	0.528
Proline	4	10.05±9.81	7	4.45±7.19	0.653
Glutamine	7	25.45±8.69	9	13.44±11.93	0.455
Ammonia	7	13.92±9.08	9	2.47±7.08	0.179
Alanine	7	14.97±1.56	9	20.64±7.72	0.679
Glycine	7	30.04±1.14	9	28.52±11.24	0.926
Cystine	7	20.82±0.69	8	7.23±10.54	0.329
Arginine	7	21.68±7.09	9	34.73±9.06	0.485
Serine	7	25.40±3.22	9	38.71±9.36	0.836
Tyrosine	7	24.90±0.66	9	27.44±4.56	0.815

Table 3 Relationship between A-V amino acid uptake rates and Dukes stage in colon cancer

Amino acid	Dukes stage B		Dukes stage C or D		*P* value
	Sample number	R1 (%)	Sample number	R1 (%)	
Threonine	10	35.75±8.85	6	45.10±7.10	0.476
Phenylalanine	10	24.26±7.02	6	29.60±6.47	0.616
Leucine	10	29.73±9.68	6	47.93±4.07	0.184
Isoleucine	9	22.74±10.23	6	42.47±4.41	0.158
Methionine	10	2.44±6.39	6	26.72±8.30	0.036
Valine	10	34.85±9.53	6	31.15±3.37	0.776
Lysine	10	20.10±11.03	6	34.34±4.90	0.356
Histidine	10	25.32±8.83	6	37.35±5.08	0.341
Proline	6	-2.70±6.43	5	17.50±7.25	0.066
Glutamine	10	26.30±9.92	6	6.02±10.95	0.209
Ammonia	10	5.44±7.74	6	3.47±10.07	0.879
Alanine	10	21.57±9.65	6	12.50±6.66	0.516
Glycine	10	29.37±8.65	6	28.87±15.91	0.976
Cystine	9	11.46±10.67	6	16.73±6.03	0.715
Arginine	10	23.12±13.84	6	38.85±4.80	0.409
Serine	10	34.32±9.75	6	42.16±12.82	0.632
Tyrosine	10	23.08±7.78	6	31.74±4.30	0.432

Relationships between the uptake rates of amino acids and the size of tumor mass or the Dukes stage are shown in Table 2 and Table 3.

DISCUSSION

Studies have noted the amino acid uptake changes in blood and tumor tissue, but the results were controversial[8,11-13]. More factors, such as ages, food intake, consumption and digestion, liver and kidney functions, could influence the amino acid concentrations in serum, and different sample treatment was also confirmed as an important factor[14-17]. In our study, we compared the amino acid concentrations in cancer tissue with those in normal colon as self-control to eliminate the factors that influenced the results.

Tumor cells needed more glucose and amino acids than normal body cells[1]. Limited data were found in *in vivo* studies. We analyzed the amino acid uptake rates in cancer tissue and revealed that the uptake rates of amino acids, especially EAAs, ketogenic, ketogenic and glucogenic amino acids, but not methionine, were significantly higher than those in normal tissue, suggesting that colon cancer might need more of these amino acids.

Protein synthesis was more active in tumor tissue[18-21], and enzymes such as protein kinase were more active in hepatic cancer cells than those in normal cells. Michael *et al*. demonstrated that the protein concentration in cancer tissue (hepatic cancer, digestive cancer and breast cancer) was elevated continuously with tumor progression. Hagmuller *et al*.[22] revealed that, not only the protein synthesis, but the uptake of EAAs and branched-chain amino acids (BAAs) in cancer tissue were more significant than those in arm tissue, as in our study. Khirallah *et al*. suggested that the sources of amino acids in cell protein synthesis came from the plasma and cell protein degradation, though it was not clear which would be the major source. According to our study, amino acids, especially EAAs, might come from the plasma because colon cancer cells were inadequate to synthesize EAAs.

Asparagic acid, glycine, glutamine, folinic acid and ammonia were the basic substrates in pyrimidine and purine nucleosides

synthesis[23]. Our data showed that asparagic acid was too low to be detected in most patients, which might be due to its total utilization in nucleosides synthesis, as was reported by Norton[6]. Methionine is one of the important amino acids in cancer metabolism, and total parenteral nutrition (TPN) with cystine and methionine deficiency has been shown to decrease the tumor growth by inhibiting DNA and RNA synthesis in cancer cells[24,25]. Our data showed that the cystine and methionine uptake rates in cancer tissue were not higher than those in normal tissue, it might be resulted from its repetitive utilization in methylation by s-ademethionine circulation. On the other hand, lower methylation in nucleosides metabolism might also decrease the cystine/methionine requirement in tumor.

Tumor growth consumes a large amount of energy. Glycolysis was ascertained as the major source of energy especially in archaeocytes and poorly differentiated cells because the enzymes in glycolysis in tumor tissue were elevated, which increased the lactic acid concentration and reduced the pH value. Because a little adenosine triphosphate (ATP) could be released by glycolysis, a lot of glucose should be consumed to get adequate energy. Most studies showed that alanine, glycine and glutamine might be involved in glyconeogenesis for their concentrations in blood were higher than the other amino acids. On the other hand, liver glyconeogenesis increased in tumor tissue, and the enzymes involved in glyconeogenesis were more activated, though they were lower in liver cancer than in normal tissue in some studies[1,26]. The glucogenic amino acid concentrations were not obviously changed in tumor tissue in our study, though the uptake rates were higher than those in normal tissue, suggesting these amino acids were utilized during glyconeogenesis.

Aerobic metabolism was present in tumor tissue. Whether any enzyme deficiency occurs in tumor aerobic metabolism is still controversial. Kern *et al*.[27] reported that the fat, but not the liver starch, was the primary substrate in energy metabolism when fasting. Compared with anaerobic metabolism, it could produce eighteen to nineteen times of ATP. The amino acid concentrations in colon cancer tissue (threonine, isoleucine, leucine, phenylalanine, proline, tyrosine and lysine) were higher than those in normal tissue in our study, suggesting that tumor colon tissue utilized these amino acids as a fuel to get more energy in Krebs cycle. There might be a more active and flawless aerobic metabolism in colon cancer tissue, and further studies should be conducted.

It seems that the poorer the cell differentiation, the more elevated ability the more amino acid uptake in tumor. The samples we selected in our study were moderately differentiated globular adenocarcinomas. It was difficult to reveal the differences according to their differentiation, and more samples are needed in further study. Several studies[28-32] demonstrated that there was a correlation between the amino acid concentrations and the tumor volume. On the contrary, in our study, there was no obvious correlation between the size of tumor and the uptake rates of amino acids in tumor tissue, which might probably due to tumor necrosis or lower metabolism. The amino acid uptake rates of patients in Dukes stage C or D were higher than those in Dukes stage B, but only methionine had statistical significance.

The uptake rates of EAAs (methionine and lysine) and NEAAs (glutamine, glycine, alanine, cystine, arginine, proline and ammonia) were negative in normal colon tissue, suggesting that normal colon tissue has the ability to synthesize these amino acids or produce them by tissue protein degradation. Studies[26,33] demonstrated that malignant neoplasms had the ability to enhance the degradation of proteins in surrounding normal tissue or muscles and absorb some part of amino acids to glyconeogenesis, though its function was ignored in general conditions. Whether it is significant in short gut patients needs further studies.

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