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FEATURES OF TRYPTOPHAN METABOLISM IN PATIENTS WITH COLORECTAL CANCER.

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Colon (colorectal cancer - colorectal cancer) is one of the leading places in the structure of the cancer incidence of gastro-intestinal tract. According to numerous publications in recent years throughout recorded steady growth of this disease (1, 2, 3, 4). Parallel increase in the incidence of colorectal cancer, increases the frequency of its complicated forms, which is more than 50% (5, 6, 7). Mortality due to the formation of colorectal cancer in the United States and Western Europe is second only to breast cancer in women, the third - after prostate cancer and lung in men. Noteworthy that 38% of colorectal cancer falls on the sigmoid, 22% - ascending colon, 16% - transverse colon, 7% - descending colon and 15% - rectum. About 50% of colorectal cancer have cancer of the colon, where its main forms are complicated by obstructive ileus (85%), perifocal inflammation (12-35%), perforation (2-27%) and bleeding (1, 2, 3 and 4).

Tryptophan is an essential amino acid required for biosynthesis of proteins, serotonin, and melatonin in the brain and other tissues (1). In mammals, however, most of the tryptophan derived from the diet is metabolized via the kynurenine pathway (2). Abnormal tryptophan oxidation along this pathway is an important mechanism for modulation of tumor cell proliferation and immunoresistance, mainly via the initial and rate-limiting step catalyzed by indoleamine 2,3-dioxygenase (IDO) (3,4). Induction of IDO leads to local tryptophan depletion, thus inhibiting cell growth in some malignant tumors (5,6). On the other hand, enhanced IDO activity in tumors may also exert a potent immunosuppressive effect by blocking T-lymphocyte proliferation, thus diminishing T-cell–mediated tumor rejection (3,4,7). Thus, manipulation of tryptophan metabolism via the kynurenine pathway may have important implications in tumor pharmacotherapy. However, it is not always clear whether IDO activity should be enhanced or inhibited to suppress tumor growth in specific tumors.

Recent studies have consistently shown high expression of IDO in a variety of human tumors, including lung tumors (3,8–14). Several of these studies demonstrated that high expression of IDO was associated with reduced survival (9–11,14). In vivo detection of abnormal tryptophan transport and metabolism via the kynurenine pathway could be an important tool in identifying patients amenable to immunotherapy targeting tryptophan metabolism in tumors and monitoring therapeutic effects. The PET radiotracer α-11C-methyl-l-tryptophan (AMT) is well suited for such studies; AMT is not a substrate for protein synthesis (15) but can be metabolized by IDO because of the low substrate specificity of this enzyme (16). Our previous studies detected a high concentration of quinolinic acid, a metabolite of the kynurenine pathway, in resected tubers showing increased AMT uptake on PET in children with tuberous

sclerosis (17). In our recent studies of brain tumors, increased AMT uptake associated with expression of IDO was found in human gliomas and glioneuronal tumors on PET (12,18).

The purpose of the present study was to explore the clinical use of AMT PET in extracerebral tumors. We selected thoracic tumors for this pilot study because lung tumors express IDO (9,13); dynamic AMT PET of such tumors can conveniently include the heart in the field of view, thus allowing us to obtain arterial blood input function from the left ventricle of the heart for a full kinetic analysis; and lung tissue shows low background for AMT transport and metabolism. The overall goal of this study was to establish the feasibility of AMT PET for detecting altered tryptophan metabolism in extracerebral (primary and metastatic thoracic) tumors. Specific goals of the study were to explore whether AMT PET detects increased transport or metabolic rate in lung tumors, as compared with unaffected lung tissue, and whether different tumor types showl different AMT transport or metabolic rates; determine whether estimation of AMT transport and metabolic rates could be achieved without arterial blood sampling, using a simplified graphical approach (19).

Purpose of the work was to study the dynamics of exchange of the essential amino acid - L-tryptophan in patients with colorectal cancer and the rationale for monitoring criteria significant indicators of early diagnosis of colon cancer pathology and optimization of pathogenetic therapy.

MATERIALS AND METHODS

239 patients at the age from 35 till 76 years with the established diagnosis of colorectal cancer (CRC) were examined and treated using clinical tools and clinical-morphological methods. According to localization of pathological process the rectum cancer (RC) was diagnosed in 54 patients (29 men, 25 women), cancer of the sigmoid colon (SC) - in 62 patients (37 men, 25 women), a cecum cancer (CeC) - in 27 patients (15 men, 12 women), cancer of the transverse colon (TCC) - in 66 patients (48 men, 18 women), a colon cancer (CoC) - in 30 patients (17 men, 13 women). The first (I) stage of cancer was detected in 6 patients, the second (II) - in 34, the third (III) - in 161 and the fourth (IV) - in 38 oncologic patients (inoperable forms of large intestine cancer). The comparison group included 43 conditionally healthy persons of similar age and sex (23 men, 20 women).

The research program included the determination of the exchange of tryptophan in the blood serum of cancer patients as well as relatively healthy patients, the content of the amino acid tryptophan and its metabolites - serotonin, melatonin, 5-OIUK, indican, and animal activity of the enzyme tryptophan-2 ,3-dioksigenazy (TRP) and one of the products of oxidative deamination of biogenic monoamines, ammonia (NH3). Determination of ammonia in the blood serum was carried out by ion-exchange chromatography on ion exchangers. After separation of substrates on the registration number of ion exchangers NH3 was carried out on an automatic amino acid analyzer T-339 (Czechoslovakia). Tryptophan metabolites, and its metabolism - serotonin, 5-OIUK determined by Atack C, Magnusson T. (13). Melatonin has been studied by ELISA with monoclonal antibodies. For this purpose, we used a set of reagents Melatonin ELISA (Hamburg), Kat-N2 RE 54 021. On the functional state of transformation processes of amino acids in the colon under the influence of microorganisms and detoxifying the liver judged by the number of end-product exchange tryptophan - an animal indican in the blood serum of patients and healthy subjects conditionally accepted method (14, 15). It is known that L-tryptophan is the stabilizer of the enzyme TRP. Contributing to the formation of a stable conformational state, the TRP of the liver has an absolute substrate specificity with respect to L-

tryptophan, and catalyzes the irreversible reaction of a key amino acid catabolism by kinureninovomu ways to exchange with the formation of N-formilkinurenina, and later one of the key end-metabolites - OVER. This enzyme accelerates the incorporation of molecular oxygen directly into the molecule of L-tryptophan, and their reaction is catalyzed by skorostlimitiruyuschey stage conversion of the substrate. The activity was determined by the TDO Badawy A. A.-B., Evans M. (16,17). *Statistical analyses* i
Cox regression models were used to estimate the association between dietary antioxidant vitamias and carotenoids, and serum concentrations of -tocopherol, -carotene and retinol and the risk for colorectal cancer. Our analysis used follow-up time starting from randomisation and ending at diagnosis of colorectal cancer, at death, or at the end of follow-up (30 April 1995). Dietary variables were log-transformed and energy-adjusted according to the Willett residual method (Willett & Stampfer, 1986). Dietary and serum variables were entered into the models as indicator variables defined by the second through fourth quartiles among the entire cohort, with the lowest quartile as the reference group. An ordinal score variable was also created to test for dose-response relationships across levels of dietary and serum variables.

RESULTS OF RESEARCHES AND THEIR DISCUSSION

239 patients at the age from 35 till 76 years with the established diagnosis of colorectal cancer (CRC) were examined and treated using clinical tools and clinical-morphological methods. According to localization of pathological process the rectum cancer (RC) was diagnosed in 54 patients (29 men, 25 women), cancer of the sigmoid colon (SO) - in 62 patients (37 men, 25 women), a cecum cancer (CeC) - in 27 patients (15 men, 12 women), cancer of the transverse colon (TCC) - in 66 patients (48 men, 18 women), a colon cancer (CoC) - in 30 patients (17 men, 13 women). The first (I) stage of cancer was detected in 6 patients, the second (II) - in 34, the third (III) - in 161 and the fourth (IV) - in 38 oncologic patients (inoperable forms of large intestine cancer). The comparison group included 43 conditionally healthy persons of similar age and sex (23 men, 20 women).

As a result of studying the exchange of L-tryptophan found a violation of the content of key metabolites of his share in the serum of patients with colorectal cancer compared with a group of quasi-healthy patients. These changes were accompanied by a significant increase in biogenic monoamine - serotonin, a hormone, melatonin, 5-OIUK, products of oxidative deamination of amino acids and biogenic monoamines - Ammonia (NH3), as well as animal indican (Table 1).

It is known that the main, if not the only mechanism by which the activity of tryptophan-2 ,3-dioksigenazy affect the synthesis of serotonin in the body, is a change in the level of free L-tryptophan (18, 19). Studies have found increased activity of TRP and the content of L-tryptophan in the blood serum. Under these metabolic conditions opens the way of increased synthesis of coenzyme forms of NAD and NADP needed to enhance recovery synthesis, differentiation and proliferation of tissues in the process of tumor growth. Regulation of the TRP is the type of feedback the final products kinurenin pathways of L-tryptophan NAD and NADP, whereas activation of the enzyme is associated with increased levels of substrate oxidation - L-tryptophan. Positive enzyme activators are ions Cu2 TRP, hematin, ferrigem and o-aminolevulinic acid (o ALA). Hemin at the same time, is a coenzyme TRP. A significant increase in activity of the TRP provides an indication of reduced protein synthetic function in patients with cancer, and in particular violation of hemoglobin synthesis, resulting in heme oxidized by oxygen in hemin,

which is a coenzyme of the enzyme activator, and on the other hand - the oxidized form of heme (hemin) inhibits the activity of mitochondrial enzyme o-aminolevulenatsintazy, which catalyzes the first reaction of heme synthesis of succinyl-CoA and glycine,!©--aminolevulinic acid.

The observed changes in the exchange of L-tryptophan and the activity of TAR, namely, their rise in serum may indicate a violation of paired metabolic processes associated with catabolic and anabolic transformations of proteins, including hemoglobin, often accompanied by the development of the anemic state in tumor lesions of the colon , which is due primarily to increased protein decomposition. Most of the metabolite of L-tryptophan, biogenic monoamine - serotonin undergoes oxidative deamination to form 5-OIUK and NH3, in this case, the conversion of L-tryptophan may be associated with the synthesis of melatonin, the levels which were significantly lower in patients with colorectal cancer. Serotonin is intermediate between hormones and neurotransmitters. It narrows the arterioles and raises blood pressure, increases peristalsis, exerts antidiuretic action. In the central nervous system, serotonin acts as a mediator and is a source of synthesis in the pineal gland hormone - melatonin. Literary sources indicate that melatonin in the neonatal period of development affects the differentiation of the brain centers that control the function of the gonads and adrenal glands in a "critical" period of development (9). Melatonin plays an important role in the mechanisms of hypothalamic neuroendocrine regulation of anterior pituitary and peripheral endocrine glands (18, 19). It inhibits the secretion of gonadotropins in the hypothalamus and causes the antagonistic relationship between the body and the gonads, as a physiological brake of premature puberty. He owns a significant role in the mechanism of "biological clock", the frequency of *University of Cambridge 800 years 1209-2009*

activation and inhibition functions of the body at different times of day, seasons, years, etc. (18, 19).

The results of the dynamics serotanina and melatonin showed significant dysfunction of the neuroendocrine system in the regulation of structural and metabolic processes and mechanisms of development of colon cancer pathology.

The level of one of the end products of metabolism amino acid tryptophan - an animal indican was significantly elevated in patients with third and fourth stage of the disease, which confirms the formation of the final increase in the toxic breakdown product of L-tryptophan - indole. These data reveal a violation of the structural and metabolic processes that are associated with digestion of proteins on the background of changes in cultural and morphological properties mikrobiotsinoza intestines, which are based on violation of the evacuation function of the colon, the development of putrefactive processes and dysbiosis (2, 3, 8). However, studies show activation of liver detoxification functions of hepatocytes, as evidenced by an increase in serum metabolite of indole bound in the form of efirosemoy acid with potassium or sodium (Indican). It is known that one of the exchange of metabolites of L-tryptophan, is a 3-gidroksiantranilovaya acid, which has antioxidant properties. It is distinguished by the ability to restore a-tocopherol is associated with low-density lipoproteins (LDL). Taking into account this information, you can expect that the enhanced activation or inhibition of TAR in the malignancy of colon cancer patients can make some contribution to the formation conditions of the cooperative interaction of the oxidant-antioxidant homeostasis, which may be associated with increased free radical processes, activation of lipid peroxidation and oxidative modification of proteins that form the development of membrane pathology.

The study of the exchange of L-tryptophan in patients suffering from colon oncopathology shows deep violations of the protein, neurotransmitter and hormonal forms of exchange involving violation mikrobiotsinoza endotoxemia, and the body, confirming that there was an increase in the serum of cancer patients derived ammonia and indole-indican (Table 1).

Studies of exchange of L-tryptophan in patients with colorectal cancer at the earliest stage of tumor (which was the detection of low and my husband was 4. And two wives. Of 239patsientov) found no statistically significant changes in the dynamics of serum ammonia, indican, L - tryptophan and the enzyme activity TAR P <0.05 was observed while the dynamics of steady increase of L-tryptophan, and TAR.

The content of serotonin, 5-OIUK in the blood serum of this group of patients was significantly increased, while melatonin reduced in comparison with the conventionally healthy patients.

Analysis of the dynamics of metabolic rates of exchange amino acid L-tryptophan suggests that the criterial - important diagnostic indicators of possible cancer development process in the body, are the contents of serum serotonin, 5-OIUK and melatonin. These data strongly support a major role in the pathogenesis of neuroendocrine regulation of the formation of colon cancer. In a place with the evaluation of the content of the exchange of metabolites of L-tryptophan, can conclusion about the prognostic stages of tumor development, which is accompanied with more severe forms of malignant growth, the accumulation in the blood serum of toxic metabolites - indole, ammonia, etc., which is an indicator of adverse homeostatic functions of the body.

I

Indicators of exchange of L-tryptophan in patients with colorectal cancer of the intestine

Indicators	Group supervision, sex, stage of disease (M ± m)						Group of conditionally healthy (M±m)	
	Men			Women			Men (n=23)	Women (n=20)
	2"(n=18)	3"(n=104)	4"(n=20)	2*(n=l6)	3"(ii=57)	4"(n=18)		
L-tryptophan (mc/1)	* 59,3±2,6	68,5±3,1	* 77,8±3,6	* 63,7±2,2	71,5±3,4	# 74,9±2,6	52,4±2,5	50,8±3,3
Serotonin (mc/1)	* 3,42±0,12	* 4,83±0,35	* 5,74±0,48	* 3,60±0,2 4	• 4,68±0,30	* 6,10±0,3	0,67±0,03	0,54±0,04
5- OIUK (mc/1)	* 0,74±0,06	* I,16±0,14	* 2,18±0,23	*• 0,65±0,0 8	* 0,97±0,18	# 1,86±0,2	0,34±0,014	0,42±0,02
Melatonin (pg/L)	89,4±3,6'	61,8±5,7	44,3±3,6	83,7±4,2	* 65,8±5,4	* 46,3±4,7	176,5±6,3	184,2±7,2
Indican (mc/1)	* 6,2±0,34	* 7,8±0,46	* 8,5±0,66	• 5,7±0,23	* 6,8±0,35	• 7,9±0,54	1,85±0,24	2,38±0,27
Ammonia (nmol / L)	* 37,2±2,6	* 49,8±3,6	* 58,7±3,2	• 40,6±2,3	* 47,5*2,8	* 59,3±4,3	2I,6±I,38	24,2±I,96
TAR (nmol kynurenine / mg» I hour protein	• 50,4±2,7	* 61,8±2,3	* 70,6±3,5	* 48,6±2,8	• 57,6*3,1	* 66,2±4,1	* 37,5±2,3	• 35,8±3,4

Thus, the study obmela amino acids and L-tryptophan can objectively confirm the stage of development of cancer and to monitor the process in the treatment of patients with colorectal cancer. Monitoring indicators are: the definition in the blood serum content of L-tryptophan, serotonin, melatonin, 5-OIUK, indican, ammonia and the enzyme activity TAR, which reflect one of the important links in the structure-metabolic disturbances in the mechanisms of formation of colon cancer pathology. Optimization of the pathogenetic therapy of colorectal cancer should include a range of therapeutic interventions aimed at normalization of the neuroendocrine regulation of metabolism of L-tryptophan, detoxification, increased antioxidant protection and inhibition of oxidative stress, improving immunological resistance in combination with surgical and chemotherapeutic effects. Monitor the effectiveness of therapeutic measures can be implemented to change the dynamics of exchange of the amino acid metabolite L-tryptophan, which is of great prognostic significance of the outcome of the disease and recovery.

REFERENCES

1 .Peters JC.| Tryptophan nutrition and metabolism: an overview. Adv Exp Med Biol. 1991;294:345-358.

2. Schwarcz R, Pellicciari R. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J Pharmacol Exp Ther. 2002;303:1-10.

3. Uyttenhove C, Pilotte L, Theate 1, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9:1269-1274.

4. Munn DH, Mellor AL. IDO and tolerance to tumors. Trends Mol Med. 2004;10:15-18.

5.0zaki Y, Edelstein MP, Duch DS. Induction of indoleamine 2,3-dioxygenase: a mechanism of the antitumor activity of interferon gamma. Proc Natl Acad Sci USA. 1988;85:1242-1246.

6.Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today. 1999;20:469^173.

7. Friberg M, Jennings R, Alsarraj M, et al. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int J Cancer. 2002;101:151-155.

8. Travers MT, Gow IF, Barber MC, Thomson J, Sherman DB. Indoleamine 2,3-dioxygenase activity and l-tryptophan transport in human breast cancer cells. Biochim Biophys Acta. 2004;1661:106-112.

9.Astigiano S, Morandi B, Costa R, et al. Eosinophil granulocytes *account* for indol eamine 2,3 -dioxygenase-mediated immune escape in human non-small cell lung cancer. Neoplasia. 2005;7:390-396.

10. Okamoto A, Nikaido T, Ochiai K, et al. Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. Clin Cancer Res. 2005;11:6030-6039.

11. Brandacher G, Perathoner A, Ladurner R, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin Cancer Res. 2006;12:1144-1151.

12. Batista CEA, Juhasz C, Muzik O, et al. Imaging correlates of differential expression of indoleamine 2,3-dioxygenase in human brain tumors. Mol Imaging Biol. In press.

13.Karanikas V, Zamanakou M, Kerenidi T, et al. Indoleamine 2,3-dioxygenase (IDO) expression in lung cancer. Cancer Biol Ther. 2007;6:1258-1262.

14. Ino K, Yamamoto E, Shibata K, et al. Inverse correlation between tumoral indoleamine 2,3-dioxygenase expression and tumor-infiltrating lymphocytes in endometrial cancer: its association with disease progression and survival. Clin Cancer Res. 2008;14:2310-2317.

15. Tohyama Y, Takahashi S, Merid MF, Watanabe A, Diksic M. The inhibition of tryptophan hydroxylase, not protein synthesis, reduces the brain trapping of alpha-methyl-1-tryptophan: an autoradiographic study. Neurochem Int. 2002;40:603-610.

16. Shimizu T, Nomiyama S, Hirata F, Hayaishi O. Indoleamine 2,3-dioxygenase: purification and some properties. J Biol Chem. 1978;253:4700-4706.

17. Chugani DC, Muzik O. a-[C-11]methyl-l-tryptophan PET maps brain serotonin synthesis and kynurenine pathway metabolism. J Cereb Blood Flow Metab. 2000;20:2-9.

18. Juhasz C, Chugani DC, Muzik O, et al. In vivo uptake and metabolism of a-[HC]methyl-l-tryptophan in human brain tumors. J Cereb Blood Flow Metab. 2006;26:345-357.

19. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab. 1983;3:1-7.

20. Hamacher K, Coennen HH, Stoecklin G. Efficient stereospecific synthesis of no-carrier-added 2-[F-18]-fluoro-2-deoxy-d-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med. 1986;27:235-238.

21. Chakraborty PK, Mangner TJ, Chugani DC, Muzik O, Chugani HT. A high-yield and simplified procedure for the synthesis of a-[llC]methyl-l-tryptophan. Nucl Med Biol. 1996;23:1005-1008.

22. Muzik O, Behrendt DB, Mangner TJ, Chugani HT. Design of a protocol for quantitative brain FDG studies with PET not requiring invasive blood sampling [abstract]. J Nucl Med. 1994;35(suppl): 104P.

23. Suhonen-Polvi H, Ruotsalainen U, Kinnala A, et al. FDG-PET in early infancy: simplified quantification methods to measure cerebral glucose utilization. J Nucl Med. 1995;36:1249-1254.

24. Belsley D. Conditioning Diagnostics: Collinearity and Weak Data in Regression. New York, NY: Wiley & Sons; 1991.

25. Chugani DC, Muzik O, Chakraborty P, Mangner TJ, Chugani HT. Human brain serotonin synthesis capacity measured in vivo with <x-[C-ll]methyl-l-tryptophan. Synapse. 1998;28:33-43.

26. Vesselle H, Schmidt RA, Pugsley JM, et al. Lung cancer proliferation correlates with [F-18]fluorodeoxyglucose uptake by positron emission tomography. Clin Cancer Res. 2000;6:3837-3844.

27. Yamamoto Y, Nishiyama Y, Ishikawa S, et al. Correlation of ¡18F-FLT and 18F-FDG uptake on PET with Ki-67 immunohistochemistry in non-small cell lung cancer. Eur J Nucl Med Mol Imaging. 2007;34:1610-1616.