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GABA CONTENT AND GAD ACTIVITY IN COLON TUMORS TAKEN FROM PATIENTS WITH COLON CANCER OR FROM XENOGRAFTED HUMAN COLON CANCER CELLS GROWING AS S.C. TUMORS IN ATHYMIC nu/nu MICE

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A significantly high GABA level and GAD activity was found in human colon cancer tissue as compared with normal macroscopically unchanged human colon wall taken from the same patients. Similarly in athymic nu/nu mice transplanted with human colon adenocarcinoma cells established in *in vitro* culture (line CX-2) the high level of GABA accompanied by high GAD activity was found in subcutaneously growing tumors as compared with the unchanged colon wall and unchanged skin tissue from the same tumor bearing mice. Interestingly, the level of GAD activity in the macroscopically unchanged colon tissue of mice tranplanted with tumor cells were increased in comparison with normal colon of healthy control mice. For the skin, only GAD activity was higher in the material coming from tumor bearing mice than in the material from normal control mice, whereas GABA level was even lower in the skin of tumor inoculated mice compared with control group.An increase in GABA level and in GAD activity can perhaps reflect a local immune response to the neoplastic process. The observed direction of GABA metabolism in tumor of the colon indicates a possibility to interfere in this process using the agonists of the GABA-ergic system.

Key words: *GABA, GAD, cancer of the colon in men, human colon adenocarcinoma, thymusless nude mice.*

INTRODUCTION

It has long been attempted to determine biochemical differences between a normal cell and a cell changed by a neoplastic process, which could be specific for neoplasia and serve as a distinctive pathological marker of the tumor cell proliferation. There are some data indicating the non-specific

differences in the activity of the enzymes involved in the metabolism of certain aminoacids and polyamines in certain types of neoplasms (16, 19, 28).

Neoplastic cells in some acute lymphatic leukemias do not show any activity of asparagine synthetase and, hence, cannot produce asparagine. After this discovery, L-asparaginase, an enzyme which decomposes L-asparagine, became a potential antitumor drug in the treatment of patients with these leukemias. "Protein hunger" appeared to be a cause of cancer cells death and this treatment was one of the first examples of a specific tumor killing without "touching" the normal cells (1, 23).

There exist experimental data indicating changes of histaminase activity in the experimental tumors in mice and in human tumors such as breast cancer and melanoma (2, 5). Another biochemical difference between a normal and neoplastic cell concerns the role of polyamines. There has been observed a relationship between processes of fast cellular growth and increase of polyamines synthesis. Putrescine, spermine and spermidyne are the products of an arginine metabolism; putrescine being assumed to control growth while spermidyne to regulate cell destruction (7, 16).

Polyamine level and activity of ornithine decarboxylase and histaminase were also examined in colon cancer and in the surrounding unchanged mucous membrane. It appeared that polyamine level was significantly higher in cancer tissue than in unchanged neighbouring mucosa. The activity of ornithine decarboxylase was higher while the activity of histaminase was significantly lower in the tumor than in the unchanged intestinal mucosa (16).

Increase in the activity of some other enzymes in tumor tissue was also found for β-glucuronidase, lactate dehydrogenase and aldolase (7, 9). However, there are no literature reports on the activity of enzymes taking part in γ-aminobutyric acid (GABA) metabolism in neoplastic cell in comparison with normal tissue. Glutamine acid decarboxylase occurring as GAD I and GAD II is the key enzyme in the GABA synthesis process (3, 27). The main function of GABA in the central nervous system (CNS) is neurotransmission by either inhibition or modulation of the energetic changes in GABA-ergic neurons (18, 20). More recently it has been shown that GABA is present also outside the CNS (4, 6, 13, 24). The presence of GABA has been confirmed in the alimentary tract, lung, pancreas, sexual organs and urinary bladder (8, 21, 24). GABA-ergic neurons are distributed in intraparietal plexi of the alimentary tract, from the stomach to the colon. They act as the components of the intestinal nervous system, independent of the CNS (4, 6, 13, 21).

Hobbiger was the first to report on GABA presence in the alimentary tract and about its role as a neurotransmitter (13). GABA function is probably the same in the alimentary tract but its mechanism of action is not yet fully clarified (4). It should be emphasized that GABA released from intestinal intraparietal plexi, acts through GABA-A and GABA-B receptors, affecting

neurons secretion of a vasoactive peptide and thus affecting the peristalsis (10). The other mechanism, involving GABA-B receptors, includes inhibition of neurotransmitters release and a modulation of acetylcholine, substance P, and encephaline secretion (10, 13).

In this paper we describe our attempt to investigate the differences in GABA metabolism between colon cancer tissue and normal unchanged colon in man and in nude mice transplanted with human colon adenocarcinoma cells.

MATERIAL AND METHODS

Patients

GABA content and GAD activity was determined in the material from 31 randomly chosen patients operated on for colon cancer in the First General Surgery and Transplantology Clinic of the Medical University of Lublin in the period from 1991 to 1993. The group comprised 12 men and 19 women. The age of the men was from 24 to 72 years (average 63.4) and of the women from 45 to 78 (average 62.6).

Preoperative diagnosis of malignancy was established by histopathological examination of the biopsy specimen taken by colono- or rectoscopy. The tumor specimens and the samples from macroscopically unchanged intestinal wall (proximal to the tumor) were taken from the surgically resected part of colon (25 to 60 cm long). In the experimental group the material was taken from the tumor mass while in the control group the material originated from the full thickness of macroscopically unchanged intestinal wall from the same patient. For obvious ethical reasons, we could not use as an additional control the colon material coming from healthy donors. The samples of tissue were placed immediately on an ice plate and then stored at $-18^{\circ}C$ until the biochemical determinations.The histology of the tumor and of unchanged intestinal wall was examined after surgery by histopathological analysis of the whole preparation. Clinical staging degree was determined according to Dukes and based on the histopathology results. Histological type of the tumor was determined microscopically for each patient according to WHO by Morson and Sobin criteria (22). Clinical malignancy grade was determined according to WHO in Hermanek's modification (12). Moreover, tumor localisation as well as death rates, GABA content as well as GAD activity in neoplastic tissue were determined for each patient.

Animal material

GABA content and GAD activity was determined in the material coming from thymusless, nu/nu, female, 10—12 weeks old NCR mice. These experimental animals, because of the congenital lack of the thymus, are immunodeficient and should accept the growth of different xenogenic material including human tumors. CX-2 human colon adenocarcinoma cells were inoculated subcutaneously (s.c.) into lateral abdominal side at an amount of 0.2 ml of 25% weight suspension (about 5×10^6 cells) per mouse. 35 mice were used as an experimental group and 11 healthy mice of the same strain were used as a control group.

GABA content and GAD activity were examined in 35 tumors harvested on 21 st day after implantation. The tumor neighbouring, unchanged skin together with abdominal subcutaneous tissue and fragments of the normal colon were also taken from mice biopsied for tumor tissue and

from 11 healthy control mice. Biopsy sections were taken under general ether anaesthesia and immediately frozen in liquid nitrogen.

Determination of GABA content and GAD activity

GABA content and GAD activity in human and mouse material were determined by means of the spectrometric method described by Lowe *et al.* (17) and modified by Sutton *et al.* (29). GABA content was expressed in μg/1g of tissue and GAD activity in μg of synthetised GABA per 1 g of tissue per hour.

Statistical analysis

Biochemical findings of GABA content and GAD activity were statisitically analysed by the arithmetic mean and standard error of the SEM mean determination. The statistical significance was determined by t-Student test. Correlation between the clinical parameters (histological type of tumor, its localisation, histological malignancy grade, clinical advancement degree and death rates) and GABA content and GAD activity in the neoplastic tissue was examined by Tuke's test (variance analysis for uncrossed mean values).

RESULTS

Clinical studies

In all cancer patients, the histological examination has revealed adenocarcinoma. Determination of the histological type has shown: type I in 23 patients, type II in 7 patients and type III in 1 patient. A highly differentiated tumor (grade I) was diagnosed in 6 patients, a moderately differentiated tumor (grade II) in 24 patients and a poorly differentiated (grade III) in 1 patient. In 17 patients stage A, in 7 stage B, in 4 stage C and in 3 patients stage D was determined according to Dukes clinical stage evaluation. In 19 patients the tumor was localised in the rectum, in 8 patients in the sigmoid, in 3 cases in the transverse colon and in 1 patient in the caecum. 4 out of 31 patients died on the 15 th, 17 th, 25 th or 30 th day after surgery.

As shown in *Table 1* the level of GABA in the tumor mass was over twice as high as the level in the surrounding, macroscopically unchanged colon wall. GAD enzyme activity followed GABA level pattern being more than twice higher in the tumor tissue than in macroscopically unchanged colon wall. Interestingly, GAD activity in the tumor tissue of 4 patients who died after operation was significantly higher than that of patients who survived (*Table 2*). There were no statistically significant differences in GABA content and GAD activity in the tumor tissue depending on histological type, malignancy grade estimation, clinical advancement and tumor localisation (data not shown).

Table 1. GABA content and GAD activity in colon tumors and unchanged colon wall from the surgically treated patients.

Tissue	N	GABA μg/1 g fresh tissue X ± SE	GAD μgGABA/1 g fresh tissue/1 hour X ± SE
1. Neighbouring unchanged colon wall	31	204.2 ± 20.3	111.5 ± 6.4
2. Tumor	31	498.8 ± 60.7 *	258.2 ± 11.3 *

* $p < 0.001$ in comparison with 1.

Table 2. GAD activity in the neoplastic tissue of patients who survived surgery and who died after surgery.

Patients	N	GAD μg GABA/1 g fresh tissue/1 hour
1. Survived	27	231.4 ± 9.8
2. Died	4	346,7 ± 64.5 *

* $p < 0.005$ in comparison with 1.

GABA content and GAD activity in CX-2 human colon adenocarcinoma cell line growing in nude mice

A significant increase in GABA level was found in tumor tissue (CX-2) as compared with skin, subcutaneous tissue and normal colon tissue tumor bearing mice. GABA level in the normal colon wall did not differ from the level in the skin with subcutaneus tissue tumor bearing mice. A marked increase of GABA level was found in CX-2 tumor as compared with the level both in the skin with subcutaneous tissue and in the colon wall of control healthy mice. No changes in GABA level between the skin with subcutaneous tissue and the unchanged colon wall from mice bearing CX-2 compared with the skin and colon healthy control mice were observed (*Table 3*).

The highest GAD activity was observed in CX-2 tumor tissue as compared with skin, subcutaneous tissue and normal colon tissue tumor bearing mice. GAD activity in the normal mucose colon wall was significantly higher than in the skin with subcutaneous tissue tumor bearig mice. A marked increase of GAD level was found in CX-2 tumor as compared with its level both in the skin, subcutaneous tissue and in the colon wall control healthy mice.There was no difference in GAD activity between the skin with subcutaneous tissue of healthy mice and the skin with subcutaneous tissue from tumor bearing mice,

while this activity was significantly higher in macroscopically unchanged colon wall from tumor bearing mice as compared with colon wall control healthy mice (*Table 3*). The normal colon tissue served as a control in a view of fact that this is natural site of colon cancer growth in men, while skin and subcutaneous tissue as a site neoplastic growth of these cells in mice.

Table 3. GABA content and GAD activity in xenografted s.c. tumors (CX-2) from athymic mice.

Tissue	Group	N	GABA μg/1 g fresh tissue X ± SE	GAD μg GABA/1 g fresh tissue/1 hour X ± SE
1. Colon	a) control	11	361.4 ± 21.9	521.1 ± 18.2
	b) tumor bearing mice	35	385.9 ± 12.6	606.6 ± 14.2 °
2. Unchanged skin and subcutaneous tissue	a) control	11	426.7 ± 19.8	413.2 ± 27.1
	b) tumor bearing mice	35	388.9 ± 21.9	468.9 ± 15.5
3. adencarcinoma cells (CX-2)	tumor bearing mice	35	682.0 ± 32.5 *	662.4 ± 20.3 *

* $p < 0.001$ in comparison with 1.b and 2.b)
° $p < 0.001$ in comparison with 1a.

DISCUSSION

Available literature data do not provide any information on γ-aminobutyric acid metabolism in neoplastic tissue. Our studies have shown a higher GABA level in colon cancer tissue as compared with the content of this aminoacid in normal colon wall. Glutamic acid decarboxylase (GAD) activity was also significantly higher in the tumor than in the surrounding unchanged tissue. These findings correlate with the results from our experimental material. In mice with transplanted human CX-2 colon adenocarcinoma cell line, the GABA concentration and GAD activity were considerably higher in tumor tissue than in unchanged material from the skin and colon. GAD, as a basic enzyme in the process of GABA synthesis, acts mainly in anaerobic conditions while the main enzyme decomposing GABA (GABA transaminase), is active in aerobic environment (11, 14, 15). The decrease of GABA catabolism and increase of GAD activity in the brain hypoxia of people who died suddenly in comparison with the chronically ill was observed (15). It should be emphasized that GAD activity in tumor tissue was higher in 4 patients who died after operation from septic shock in

comparison with enzyme activity in neoplastic tissue other patients. Therefore, one can wonder if the observed differences do not result from blood supply disturbances in the tumor tissue. The results of our investigations performed in nude mice are not consistent with this conclusion. An increase of GAD activity, not only in the tumor tissue but also at the natural site of growth of this cancer in men, in the colon wall, was observed.

Basing on the analysis of the obtained results, it seems that GABA-ergic system activity may be connected with the process of oncogenesis in the alimentary tract. On the one hand, the observed changes can favour the neoplastic process, but on the other hand an increase of GABA content and GAD activity can express normal tissue response to the tumor development.

Such a suggestion is supported by the recently published data which point to the protective effect of agonists of GABA-A and GABA-B receptors against the chemical induction of some tumors growth in the animals (25, 26). Muscimol, an agonist of GABA-A receptor, shows protective action in stomach cancer induction in rats by N-nitro-N-nitrosoguanidine (26). Since muscimol is a highly toxic agent, it could not be used in clinical practice. However, baclophen, an agonist of GABA-B receptor reveals protective action against an induction of the colon cancer in rats and is also used in the treatment of diseases associated with an increased tension of the skeletal muscles in clinics (25). Therefore, baclophen could be potentially used in the clinical trials in cancer prevention in patients at high risk of colon cancer or as a supplement to classical antitumor therapy.

The observed direction of GABA metabolism in the neoplastic tissue of the colon suggests a possibility of tumor growth modulation by the agonists of GABA-ergic system.

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