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STUDYING THE EFFECT OF HYPOTHYROISIS AND THYROTOXICOSIS ON THE DEVELOPMENT OF CARCINOGENESIS IN EXPERIMENTAL ANIMALS

M. A. Ismayilova, *Samarkand State Universitynamed after Sharof Rashidov*
***B.M.Bazarov,** *Samarkand State Universitynamed after Sharof Rashidov*
***e-mail:** bbozorov@samdu.uz*

Abstract. In recent years, studies have appeared whose results suggest that the effect of thyroid hormones on various metabolic processes occurring in organs and tissues of the body can lead to the induction of apoptosis and a decrease in proliferative activity in cells of various etiologies. At the same time, there are data on the participation of L-thyroxine in the occurrence of malignant neoplasms in violation of the secretion of this hormone by the thyroid gland. Thus, in diseases of the mammary gland, including breast cancer, there is a cascade of disorders of the hormonal status of the body of women, consisting of changes in the secretion of steroid hormones and a decrease in the expression of estrogen and progesterone receptors, which is based on pathologies of the thyroid gland, leading to a significant decrease in production of L-thyroxine.

Keywords. Thyroxine, thyroglobulin, thyroid gland, L-thyroxine, carcinogenesis, hypothyroidism, thyrotoxicosis, apoptosis.

Introduction. In recent years, studies have appeared whose results suggest that the effect of thyroid hormones on various metabolic processes occurring in organs and tissues of the body can lead to the induction of apoptosis and a decrease in proliferative activity in cells of various etiologies [1]. At the same time, there are data on the participation of L-thyroxine in the occurrence of malignant neoplasms in violation of the secretion of this hormone by the thyroid gland. Thus, in diseases of the mammary gland, including breast cancer, there is a cascade of disorders of the hormonal status of the body of women, consisting of changes in the secretion of steroid hormones and a decrease in the expression of estrogen and progesterone receptors, which is based on pathologies of the thyroid gland, leading to a significant decrease in production of L-thyroxine [2, 3, 4, 5].

The study of the peculiarities of the mechanism of regulation of apoptosis and proliferation of tumor cells by thyroid hormones is impossible without revealing the signal relationships in the "thyroid hormones-immunity-cancer cell" system. Analysis of literary sources allows us to speak about the significant effect of thyroid hormones on the functioning of the immune system in the body. Our study of the influence of the status of thyroid hormones on thymus involution under conditions of an experimental carcinosystem allows us to reveal some features of the mechanism of suppression of the growth of experimental tumors during thyroid induction. The created model conditions for hypothyroidism and thyrotoxicosis made it possible to assess the degree of influence of the thyroid status on the functioning of the immune system under conditions of tumor growth and to establish the morphological and functional features of immunocompetent cells in all major organs of the formation of the body's immune response to a carcinogenic attack.

Summarizing the obtained results, we can conclude the following.

The course of carcinogenesis, in the case of induction of model hypothyroidism and thyrotoxicosis, proceeds differently. Deficiency of thyroid hormones leads to a significant increase in body weight of experimental animals, does not cause inhibition of tumor growth, and does not statistically significantly reduce the proliferation of cancer tissue. On the contrary, an excess of thyroid hormones does not allow the development of the process of carcinogenesis, inhibits the proliferation of cancerous tissue and induces apoptosis in tumor cells. In the case of thyrotoxicosis induction, the tumor tissue regresses, and the degree of regression is significant - tumor growth index (TGI) = 2.34. A statistically significant decrease in the mass and volume of the tumor tissue was observed only in this group: inhibition of tumor growth by weight was 98.16%, in fact, the development of carcinogenesis in these animals did not occur. This can be explained by the factors of the influence of thyroid hormones on various metabolic processes occurring in the organs and tissues of the body, which leads to a change in proliferative activity in cells of various etiologies. At the same time, the tumor tissue in the control group of animals progressed; increased its cell population.

Materials and methods. When conducting the studies described in this paper, l-thyroxine produced by Berlin-Chemie (Germany) was used.

Table 1 shows data on the type and number of animals used in the experimental work. Animals weighing 20-22 g were kept in plastic cages (6 per cage) under standardized conditions of relative humidity (50-60%), temperature (22°C) and light regime (12 hours of darkness and light). Mice received standard commercial chow and drinking water ab libitum.

All painful manipulations with laboratory animals were performed under ether anesthesia and in strict accordance with the Declaration of Helsinki on the Humane Treatment of Animals (World Medical Association, Edinburgh, 2000).

Table 1.
Type and number of animals used in experimental work

Purpose of the experiment	Used animals			Number animals
	Kind	Line	Gender	
Determination of antitumor activity on the AKATOL strain	mouse	BALB/c	males	40
Determination of antiproliferative activity on the AKATOL strain	mouse	BALB/c	males	30
Study of thymus involution during carcinogenesis under conditions of experimental hypothyroidism and thyrotoxicosis	mouse	BALB/c	males	40
Study of the concentration of endogenous l-thyroxine under conditions of experimental carcinogenesis	mouse	BALB/c	males	40
Maintaining transplantable tumor strain AKATOL	mouse	BALB/c	males	26
Total mice used				176

The average tumor volume was found by the formula[6]:

$$V_{cp} = \frac{\pi}{6} \cdot ABC$$

where,A,B.C – length, width and height of the tumor; V_{cp}- mean tumor volume in cm^3.

Animals were sacrificed under ether anesthesia no later than 16 days after tumor implantation. The control group served as a group of animals with the introduction of solutions used to dissolve the test substances - physiological saline.

The percentage of inhibition of tumor growth was determined at the end of the experiment by the formula:

$$T\% = B_K \cdot 100 \frac{B_K - B_0}{}$$

where,B_K - average tumor mass in animals of the control group,

B_0 - average tumor mass in animals of the experimental group.

Results and discussion. In an in vivo experiment, the effect of hypothyroidism and thyrotoxicosis on the development of carcinogenesis in experimental animals was studied on the model of a tumor strain of colon adenocarcinoma AKATOL. The experimental animals were divided into 4 groups: Group I - the animals underwent thyroidectomy (removal of the thyroid gland), which caused hypothyroidism, i.e. lack of T4 in the body; Group II - animals received T4 per os in a high (5 mg/kg) dose, which led to the development of thyrotoxicosis, i.e. excess T4 in the body; group III - control, tumor-bearing animals were not exposed to any effect; Group IV - intact healthy animals that did not undergo tumor implantation.

Table 2 presents data on changes in the weight of animals by the day the experiment ended (monitoring was carried out from the day of tumor implantation). The greatest weight gain during the experiment period (21 days) was observed in experimental animals of group I - the weight of mice increased by 87.22%. Such a significant increase in body weight is due to the fact that a deficiency of thyroid hormones causes significant lipid metabolism disorders, which are manifested in an increase in blood levels of total lipids, cholesterol, phospholipids, triglycerides and a decrease in non-esterified fatty acids. The progression of these changes causes the weakening of lipolysis and the mobilization of fatty acids, inhibition of the activity of lipoprotein lipase [8].

In group II, where the model system of thyrotoxicosis was implemented, during the period of the experiment (21 days) a decrease in body weight of experimental animals was observed - the weight of mice decreased by 5.07%. The molecular mechanism by which weight loss occurs in thyrotoxicosis has not been precisely established, but we, based on previously published data [7], believe that T4 increases the activity of sodium-potassium adenosine triphosphatase (ATPase) in many tissues, and this suggests that the efficiency of the energy received from food is depreciated due to the useless cycle of adenosine triphosphate (ATP) synthesis and the loss of part of the energy in the form of heat. Another cause of weight loss due to increased metabolic rate is pheochromocytoma, the inducing factor in which is the release of catecholamines. Pituitary cachexia and adrenal insufficiency may also be associated with weight loss, mainly as a result of decreased appetite secondary to cortisol deficiency [10].

In healthy intact animals that did not undergo implantation of the AKATOL tumor (group IV), an increase in body weight by 27.19% was observed during the experiment period (21 days). During the same period, in tumor-bearing animals of the control group III, body weight increased by 35.45%.

Table 2.
Change in body weight of mice of the BALB/c line from the day of implantation of the AKATOL tumor

Groups	Body weight, g				
	Day 0	Day 7	Day 10	Day 14	Day 21
Group I. Hypothyroidism	9,0±0,77	11,33±0,88	11,94±0,65	14,16±1,03	16,85±0,71
Group II. Thyrotoxicosis	12,43±0,44	12,34±0,52	12,30±0,76	12,00±1,1	11,83±1,18
Group III. Control - tumor-bearing animals	12,41±1,28	12,13±1,71	14,03±1,65	14,75±1,70	16,81±1,23
Group IV. Healthy animals without tumor implantation	11,4±0,84	11,44±0,64	12,93±0,77	13,03±0,91	14,50±0,99

It should be noted that in the course of carcinogenesis, a significant increase in body weight is not a favorable factor, since it can be explained both by an increase in the mass of the tumor itself and by a pathological change in the mass of a number of organs due to a high degree of exudation and neutrophilic invasion.

Table 3 presents the results of the development of carcinogenesis of the experimental ACATOL tumor in BALB/c mice with induced hypothyroidism (group I) and thyrotoxicosis (group II). A statistically significant decrease in the mass and volume of tumor tissue was observed only in group II, where mice were induced with model thyrotoxicosis by introducing high doses (5 mg/kg) of T4. Inhibition of tumor growth in this group by weight was 98.16%, in fact, the development of carcinogenesis in these animals did not occur (Fig. 1). This can be explained by the influence of T4 on various metabolic processes occurring in the organs and tissues of the body, which leads to a change in proliferative activity in cells of various etiologies. This applies both to non-transformed cells, such as cells of the immune system, mammary gland, pancreatic beta cells, and malignantly proliferating cells, in particular, breast cancer and skin lymphomas.

Table 3.
Changes in the mass and volume of the AKATOL tumor in experimental animals by the day of the end of the experiment (21 days)

Groups	**Weight tumors, g**	**Tumor volume, cm^3**	**Tumor growth inhibition (TRO), %**
Group I. Hypothyroidism	1,75±0,23	2,10±0,37	35,89
Group II. Thyrotoxicosis	0,05±0,02*	0,01±0,008*	98,16
Group III. Control - tumor-bearing animals	2,73±0,22	2,87±0,29	-

*Note: * - p<0,05*

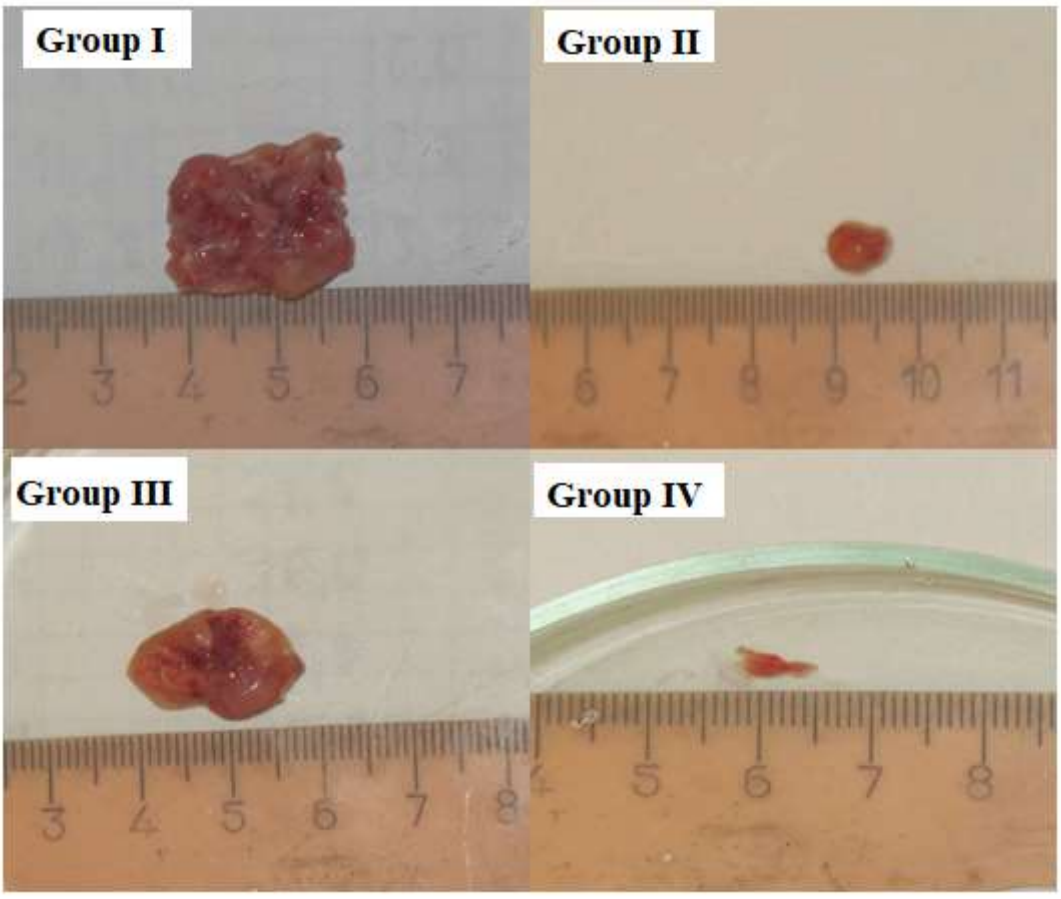

Fig. 1. Samples of the experimental tumor AKATOL implanted in BALB/c mice with induced hypothyroidism (group I) and thyrotoxicosis (group II), group III - control

Thus, T4 is able to stimulate the proliferative activity of immunocompetent cells, thus increasing the body's immune resistance to tumor progression, and inhibit carcinogenesis. This is also confirmed by data on the participation of T4 in the occurrence of malignant neoplasms in violation of the secretion of this hormone by the thyroid gland. So, in diseases of the breast, including breast cancer, there is a cascade of disorders of the hormonal

status of women, consisting of changes in the secretion of steroid hormones and a decrease in the expression of estrogen and progesterone receptors, which is based on thyroid pathologies, leading to a significant decrease in the production of T4 [5].

Metric data of tumor mass and volume are not always adequately able to reflect the processes of progression or regression of tumor tissue. This is due to the fact that a high degree of exudation, neutrophil invasion, zones of necrotic destruction, or high differentiation of the cellular composition, accompanying the processes of malignant transformation of body tissues, can change the mass or volume of the tumor in one direction or another. A more objective picture of the proliferative activity of tumor cells is given by counting the number of mitoses and in the tumor tissue - its mitotic index (MI).

Table 4 shows the results of determining the number of dividing cells in the AKATOL tumor tissue in BALB/c mice with induced hypothyroidism (group I) and thyrotoxicosis (group II). A statistically significant decrease in the number of mitoses in the tumor tissue was observed only in group II with model thyrotoxicosis induced in mice. The number of dividing tumor cells in tissue samples of this group was 87.72% less than in histological preparations of the control group (group III). The cellular composition of the tumor tissue of group II is highly differentiated, represented by small, medium, and, rarely, large cells (Fig. 2). The shape of cells and nuclei did not differ from those in the control group. In most nuclei, the chromatin is coarse-grained, fine-meshed, but in contrast to the control, cells with large-lumpy and condensed chromatin are more common.

Table 4.
Mitotic activity and apoptosis of tumor tissue cells in BALB/c mice with induced hypothyroidism and thyrotoxicosis

Groups	**Number of examined cells**	**MI, ‰**	**AI, ‰**
Group I. Hypothyroidism	10000	2,32±0,59	2,95±0,70
Group II. Thyrotoxicosis	4000	0,47±0,28*	1,1±0,54*
Group III. Control - tumor-bearing animals	10000	3,83±0,44	2,62±0,25

*Note: * - p<0,05*

Multinucleated cells are found in the same number as in the control, often of medium size. The number of nuclei in them is different, cells with 2-3 nuclei predominate. The cytoplasm of many cells is granular, with a basophilic granular component. The mitotic activity of the tissue is low, mainly with a pathological course of division stages, pathological metaphases are more common. In general, the studied tissue was a highly differentiated cell array with low mitotic activity, with extensive areas of necrosis, with a tendency to tumor tissue degradation (Fig. 2).

Fig. 2. Area of tumor tissue AKATOL. Group II (thyrotoxicosis). Polymorphism of tumor cells. Apoptotic cells (shown by arrow). Stained with hematoxylin and eosin. SW. OK. 10x, about. 100x.

The criterion of proliferation, reflecting the processes of physiological death of tumor cells, is the apoptotic index (AI). The importance of this parameter for constructing an adequate picture of the kinetic processes of the tumor tissue is explained by differences in the division cycle of tumor cells. In particular, during transformation, the cell acquires the ability to enter the G0 phase (the state of the cell before receiving a division stimulus) into G1 (preparation for DNA synthesis) and go through the entire division cycle without external or with weakened stimulation [4].

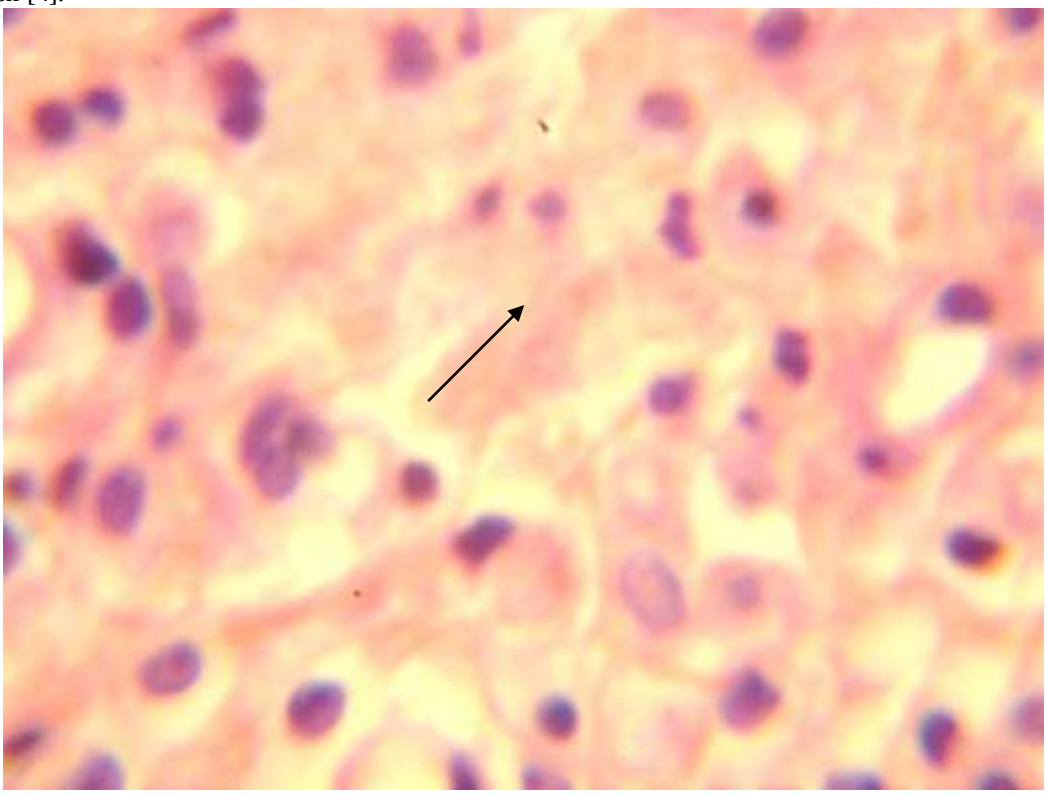

Fig. 3. Area of tumor tissue AKATOL. Group II (thyrotoxicosis). Low mitotic activity. Extensive areas of necrosis. degradation of tumor tissue. Stained with hematoxylin and eosin. SW. OK. 10x, about. 100x.

The duration of the G1+S+G2+M phases in tumor cells does not decrease compared to the norm. The mechanism of intensive unstimulated division of tumors is associated in most cases with a mutation in the proto-oncogene encoding the receptor protein on the cell membrane and making this protein permanently activated, transmitting signals for division along the chain into the cell, or with mutations in the genes of one of the intermediate proteins, which also leads to to the permanent activation of this protein and signal transmission to the next components of the chain [2]. The effect of antitumor drugs on the inhibition of the proliferation of pathological tissues should lead to an interruption in the division stimulation chain, by affecting receptors or proteins responsible for mitosis, or to changes in the cell genome. In both cases, we can talk about the activation of the mechanisms of programmed cell death or apoptosis. Therefore, when analyzing the proliferative activity of tumor tissue, it is impossible to talk about the progressive or, conversely, regressive nature of this process only taking into account the number of mitoses, since it is impossible to adequately assess absolutely the entire number of dividing cells during morphological analysis due to the transience of some phases of mitosis, and apoptotic the index, together with the mitotic index, gives an objective assessment of the processes of tumor growth or death.

Table 5.
Tumor growth index (TGI) AKATOL in BALB/c mice with induced hypothyroidism and thyrotoxicosis

Groups	**AI/MI**
Group I. Hypothyroidism	1,27
Group II. Thyrotoxicosis	2,34
Group III. Control - tumor-bearing animals	0,68

Table 5 shows the results of determining the apoptotic index (AI) in the tumor tissue AKATOL in BALB/c mice with induced hypothyroidism (group I) and thyrotoxicosis (group II). A statistically significant difference in the number of apoptotic cells in the tumor tissue was observed only in group II with model thyrotoxicosis induced in mice. In this group, the number of apoptotic cancer cells was 58.01% less than in histological preparations of the

control group (group III). However, it should be noted that in group II, the number of apoptotic cells exceeded the number of mitotically dividing cells, while in the control group of animals, the MI values exceeded the AI values, which indicates the growth of tumor tissue. Tumor tissue growth index (TGI), i.e. the ratio of the number of apoptotic cells to the number of cells in the state of division - AI / MI - allows you to evaluate the rate of regression or progression of the tumor. In the case of obtaining values less than 1.0, we have a progression of the tumor tissue, since the number of mitotically dividing cells exceeds the number of dying cells, and in the case of obtaining values AI / MI > 1.0, we have a regression of the tumor, respectively, the values themselves also show the rate of growth or death of tumor tissue.

Table 5 shows the values of RDI (AI/MI) for all experimental groups. As can be seen from the obtained values, in the case of thyrotoxicosis induction (group II), the tumor tissue regresses, while the degree of regression is significant - RDI=2.34. At the same time, the tumor tissue in the control group of animals is progressing; increases its cell population.

Conclusions. Thus, the development of carcinogenesis, in the case of induction of model hypothyroidism and thyrotoxicosis, proceeds differently. Deficiency of thyroid hormones leads to a significant increase in body weight of experimental animals, does not cause inhibition of tumor growth, and does not statistically significantly reduce the proliferation of cancer tissue. On the contrary, an excess of thyroid hormones does not allow the development of the process of carcinogenesis, inhibits the proliferation of cancerous tissue and induces apoptosis in tumor cells. Given the significant role of thyroid hormones in the regulation of the proliferation of immunocompetent cells and participation in the occurrence of malignant neoplasms in thyroid pathologies, it can be concluded that the neuroendocrine regulation of immune functions in the system "cancer cell - thyroid status - immunity".

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