

Influence by hypothyroidism on the level of sex hormones in blood and their intracrine content in the tumor in rats with Guerin's carcinoma

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Abstract

The aim of our research work was to study the level of sex steroids in blood, the tumor and the perifocal zone in rats of both sexes with Guerin's carcinoma against the background of hypothyroidism.

Materials and methods. The experiment was performed in 110 outbred rats of both sexes. Hypothyroidism was induced in animals for 30 days with Mercazolil medication, and then Guerin's carcinoma was transplanted (the main group). The reference groups included animals with an independent growth of Guerin's carcinoma and with independent hypothyroidism, as well as intact animals (the norm). On the 18th day of the tumor growth, the animals were sacrificed, and, using standard RIA kits, in the serum, the tumor homogenates and the perifocal zone, the levels of estradiol (E2), testosterone (T) and progesterone (P4) were determined.

Results. In comparison with intact animals, hypothyroidism caused an increase in the blood content of E2 in animals of both sexes by 2.2-2.4 times and T by 1.4-1.6 times, and P4 by 1.7 times only in females, but recorded was a decrease therein in males by 2.4 times. The growth of Guerin's carcinoma resulted in a 2.5-5.5-fold decrease in E2 in blood of the animals of both sexes, an increase in T by 2.1 times and P4 by 3 times in the females, but a decrease in T by 2.6 times without changing P4 in the males. In the main group, in the animals of both sexes, similarly to the processes in hypothyroidism, the level of E2 and P4 increased by 1.4-1.6 times, and in females also T by 4.4 times, compared with the intact animals.

Conclusion. Hypothyroidism and the growth of Guerin's carcinoma changed E2 in different directions in animals of both sexes the level of sex hormones in blood and shifted the steroid balance in the tumor and its perifocal zone. In the females of the main group, the saturation of the tumor with estrogens, androgens and progesterone decreased, while in the males, on the contrary, the concentration of steroids increased.

Keywords

Hypothyroidism, Guerin's carcinoma, Estradiol, Testosterone, Progesterone

Imprint

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Introduction

The regulation of the reproductive status by thyroid hormones (TH) in mammals is quite complex [1]. TH can affect sex steroids in several ways: by acting directly on the gonads, through their interaction with estradiol (E2), progesterone (P4), testosterone (T), FSH, LH and prolactin (PRL), or on the production of GnRH-releasing hormone in the hypothalamus pituitary-gonadal axis (HPG) [2, 3].

Both thyroid and steroid hormones are able to bind directly to nuclear receptors and act as transcription factors, mediating intracellular effects. In this case, continuous intracellular crosstalk is possible, which is induced by the combined action of TH and steroid hormones on target cells [4, 5]. It is known that the DNA binding site of the estrogen receptors is similar to the same binding site of the thyroid receptors: they both have the same DNA-binding sequence AGGTCA. As a result, cross-talk between the steroid hormone receptors (including the estrogen, progesterone, androgen receptors, and glucocorticoid receptors) and the thyroid hormones through incomplete elements of the hormonal response or coactivators is possible that leads

to activation or inhibition of the gene transcription that implies the proper biological effect produced by steroid and thyroid hormones, their genomic effect [1].

It turned out that the TH dysregulation in rat models with hypothyroidism does not always induce a decrease in the level of serum sex hormones, similarly to that when their increase is not always can be detected in hyperthyroidism [6].

Steroid metabolism in peripheral tissues (intracrinology) is now considered as the key way by which tissues can use hormones to respond to local physiological needs and “fine tuning” in steroid activation or inhibition as hormone receptor-dependent processes [7].

A number of studies have shown that changes in the level of hormones in the blood serum do not reflect their local content in the peripheral glands, in the tumor and its microenvironment [8]. That is, the local, or intracrine, biosynthesis and the action of sex steroids in the tumor are independent of the availability and the synthesis in other tissues and thus ensure that there is no regulation by the body.

The aim of our research work was to study the level of sex steroids in blood, the tumor and the perifocal zone in rats of both sexes with Guerin's carcinoma against the background of hypothyroidism.

The experiment was performed in white outbred rats of both sexes weighing 150–180 g. The animals were delivered by the Federal State Budgetary Institution Scientific Center for Biomedical Technologies at the Federal Medical and Biological Agency (Andreevka Branch, Moscow Region). The laboratory animals were kept under natural light conditions with free access to water and food. Work with animals was carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 86/609/EEC), as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 “Approval of the rules of laboratory practice” dated June 19, 2003, issued by the Ministry of Health of Russia. Animals were manipulated in a box in compliance with the generally accepted rules of asepsis and antiseptis. Reference: Record No. 25/129 dated 10/27/2021 prepared by our Ethics Committee.

We used in our research work a strain of Guerin's carcinoma, delivered by the Federal State Budgetary Institution “The Russian Cancer Research Center named after N.N. Blokhin” at the Ministry of Health

of Russia. Material for transplantation was obtained from donor rats on days 12–16 of the tumor growth. Transplantation of Guerin's carcinoma to animals was carried out by standard subcutaneous injection of a tumor suspension under the skin of the right shoulder blade in a volume of 0.5 ml of cell suspension diluted 1:10 in saline.

The white outbred rats of both sexes received for 30 days pharmacopoeial thyreostatic drug Merkazolil (“Akrikhin” Russia) at a daily dose of 2.5 mg/100 g of body weight (the total dose was 75 mg/100 g of body weight). The animals did not refuse to eat, gained weight, and no deterioration in the appearance of the skin and the hairline, no lethargy or drowsiness were recorded. Hypothyroidism in animals was confirmed by determining the content of total thyroxine and thyroid-stimulating hormone in the blood serum, by radioimmunoassay using standard kits (Immunotech, Czech Republic) 30 days after the thyreostatic agent medication. The animals of each sex were divided into groups comprising 15 females and 15 males: The main group was composed by the rodents after persistent hypothyroidism, and the animals therein were subcutaneously transplanted with Guerin's carcinoma.

Reference group No. 1 covered the animals with hypothyroidism.

Reference group No. 2 included the animals without hypothyroidism, with an independent growth of Guerin's carcinoma only.

The intact animals (10 females and 10 males) were used as those showing the physiological norm.

The animals were decapitated (reference group No. 2 and the main group after 18 days of growth of Guerin's carcinoma). In the blood serum of the intact rats, the animals of reference groups No. 1 and 2, as well as in the main group and in 10% tumor homogenates and in the perifocal zone in the animals of the main group and reference group No. 2, by the RIA method using standard kits (Immunotech, Czech Republic) determined were the levels of estradiol (E2), testosterone (T) and progesterone (P4).

Our statistical analysis of the results was performed using the Statistica 10.0 software package. The data obtained were analyzed for the compliance of the distribution of signs with the normal distribution law using the Shapiro-Wilk test (for small samples). Comparison of quantitative data in groups was performed using Student's t-test and Mann-Whitney. Table data are presented herein as $M \pm m$, where M is the arithmetic mean, m

is the standard error of the mean; $p < 0.05$ was taken as the level of statistical significance.

Results

Previously, we found that in female rats, hypothyroidism slowed down the increase in the volume of primary tumors of Guerin's carcinoma and prolonged their life expectancy. In males, a slight slowdown in the tumor growth was noted only up to day 14 after inoculation, then the tumor grew rapidly, and its volume did not differ from that recorded in the reference group, and no increase in life expectancy was reported [9, 10]. It was just during that period, upon expiration of 19 days of the tumor growth, when significant gender differences in the growth of Guerin's carcinoma were revealed by us, and studies of indicators in blood and tissues were carried out.

In the females of the reference group with Guerin's carcinoma, compared with the intact animals, the levels of T and P4 in blood increased by 2.1 times and 3 times, respectively, but the content of E2 decreased by 5.5 times. In the males with Guerin's carcinoma,

compared with the physiological norm, the level of E2 and T in blood decreased by 2.5 times and 2.6 times, respectively, but the content of P4 did not change.

Hypothyroidism in its independent variant (reference group No.1), compared with the intact group, induced an increase in the blood content of E2 in animals of both sexes by an average of 2.2 times and T by 16.2 times in the females and 1.4 times in the males, but however reported was a decrease in P4 by 2.4 times in the males, while it increased by 1.7 times in the females.

The main group was of particular interest. In the females of the main group, where the growth of the malignant tumor slowed down, in their blood, compared with the females with the independent tumor growth, the level of E2 sharply increased by 7.7 times and the level of T was recorded to be 2.1 times higher, while the level of P4 did not change. At the same time, compared with the indicators in the intact animals, the content of sex steroids in blood of the females of the main group was increased: E2 by 1.4 times, T by 4.4 times and P4 by 2.9 times, respectively.

Table 1

Content of sex steroids in blood of rats with Guerin's carcinoma against the background of hypothyroidism

Indicator	Intact animals	Reference No.1 Hypothyroidism	Reference No.2 Guerin's carcinoma	Main group Guerin's carcinoma against the background of hypothyroidism
FEMALES				
E2 (pg/mL)	457,9±46,6	1000±104,6 $p^1-0,0002$	83,1±7,1 $p^1-0,0000$ $p^2-0,0000$	641,9±53,4 $p^1-0,0252$ $p^2-0,0069$ $p^1-0,0000$
T (ng/mL)	0,34±0,03	5,5±0,56 $p^1-0,0000$	0,7±0,004 $p^1-0,0000$ $p^2-0,0000$	1,5±0,17 $p^1-0,0000$ $p^2-0,0001$ $p^3-0,0002$
P4 (ng/mL)	35,5±3,3	60,3±4,95 $p^1-0,0005$	106,7±6,9 $p^1-0,0000$ $p^2-0,0000$	103,7±9,5 $p^1-0,0000$ $p^2-0,0007$
MALES				
E2 (pg/mL)	273,6±26,1	660,5±54,5 $p^1-0,0000$	107,6±7,5 $p^1-0,0000$ $p^2-0,0000$	432,6±42,0 $p^1-0,0048$ $p^2-0,0038$ $p^3-0,0000$
T (ng/mL)	2,9±0,36	4,17±0,38 $p^1-0,0267$	1,1±0,12 $p^1-0,0001$ $p^2-0,0000$	2,32±0,26 $p^2-0,0009$ $p^3-0,0005$
P4 (ng/mL)	127,4±8,5	52,17±4,7 $p^1-0,0000$	128,9±5,7 $p^2-0,0000$	487,25±40,8 $p^1-0,0000$ $p^2-0,0000$ $p^3-0,0000$

Notes: significant differences compared with the following: ¹with intact animals; ²with Reference No.1; ³with Reference No.2.

In the males of the main group, there was no inhibitory effect produced by hypothyroidism on the growth of Guerin's carcinoma recorded, and the levels of E2, T and P4 in their blood were higher than in the animals of the reference group by 4 times, 2.1 times and 3.8 times, respectively. At the same time, compared with the intact animals, in the males of the main group the levels of E2 and P4 were found to be increased by 1.6 times and 3.8 times, respectively, and T did not have significant differences.

By analyzing the obtained results of the level of sex hormones in blood, we can say that in the animals of the main group, regardless of gender, the levels of E2 and P4 in the serum increased, while under the independent growth of Guerin's carcinoma under the conditions of the normal TH content (reference group) in blood of the animals of both sex, the level of E2 decreased, but in males the content of T and P4 increased, and in females T decreased and P4 did not change, compared with the physiological norm.

In this regard, as a consequence, a question arose why an increase in the concentration of E2 in blood in the animals of the main group not only did not lead to an increase in the tumor volumes, but in the females it was accompanied even by a reduction therein.

In an attempt to reveal how the change in concentrations of the sex steroids in blood corresponded to local changes in the tumor and the surrounding tissue

thereof, we studied the level of sex steroids in rats of the main group, demonstrating the growth of Guerin's carcinoma against the background of hypothyroidism, and the reference group with the standard growth of Guerin's carcinoma.

It turned out that in the female rats, where the tumor volumes in the main group were recorded to be smaller than in the reference group, despite an increased content of all sex steroids in their blood, the level of E2 in the tumor was 1.4 times lower than in the reference group, and T and P4 were found to be 1.7 times and 1.6 times higher, respectively. The ratio E2/T in the tumor in the females of the main group was 2.2 times lower than in the reference group.

In the perifocal zone in the females of the main group, the levels of E2, T and P4 were lower than those in the reference group by 2.8 times, 7.6 times and 1.8 times, respectively. The E2/T ratio also was recorded to be 1.5 times lower compared with the perifocal zone of the reference group. In the males of the main group, in their tumor samples, the levels of T and P4 were higher than those in the reference group by 6 times and 3.9 times, respectively, and the content of E2 had no differences. The E2/T ratio turned out to be 7.7 times lower.

In the perifocal zone in the males of the main group, the level of E2 exceeded the reference values by 3.5 times, while the concentrations of T and P4 were

Table 2

The level of steroid hormones in the tumor and perifocal zone (PZ) in rats with Guerin's carcinoma and hypothyroidism + Guerin's carcinoma

	E2 (pg/g tissue)	T (ng/ g tissue)	P4 (ng/ g tissue)	E2/T (arb.u.)
FEMALES				
Tumor Reference	64,8±5,6	0,3±0,04	5,7±0,46	216,4±20,3
PZ Reference	106±7,5	1,4±0,11	8,4±0,85	78,3±7,9
Tumor Main group	47,7±4,8 p1=0,0328	0,5±0,06 p1=0,0103	9±0,89 p1=0,0040	96,9±9,4 p1=0,0003
PZ Main group	38,1±4,6 P2=0,0000	0,8±0,11 P2=0,0014	1,1±0,13 P2=0,0000	51,3±4,8 P2=0,0115
MALES				
Tumor Reference	64,4±4,8	0,3±0,04	12,9±0,82	214,7±19,6
PZ Reference	13,1±0,98	1±0,09	12,3±0,9	13,1±1,4
Tumor Main group	50,4±4,5 p1=0,0468	1,8±0,15 p1=0,0000	50,5±4,8 p1=0,0000	28±2,7 p1=0,0000
PZ Main group	46,5±5,0 P2=0,0000	0,7±0,08 P2=0,0209	0,2±0,04 P2=0,0000	66,4±6,5 P2=0,0000

Notes: Significant differences compared with the following: 1 – with indicators in the tumor in the reference group; 2 – compared with the indicators in the perifocal zone in the reference group.

1.4 times and 61.5 times lower, respectively. The E2/T ratio in the perifocal zone of the males of the main group was 5.1 times higher than that in the corresponding samples of the reference group.

Discussion

Thyroid and sex hormones are able to mediate the biological effects of each other, providing multiple selectivity for the gene regulation [11, 12] that confirms the possible effect produced by the sex steroids on the tumor process against the background of the TH deficiency.

There is evidence available that estrogen metabolism may be altered as a result from the thyroid dysfunction. Thus, hypothyroidism can lead to a decrease in the metabolic clearance of estrone (E1) and excessive production of estriol (E3) [13].

Induced hypothyroidism changed the content of the sex steroids in the tumor and the perifocal zone in the animals of both sexes with transplanted Guerin's carcinoma. As a result, while in the animals of the reference group some sex differences were revealed only in the content of P4 in the tumor, the level of which was recorded to be higher in males, the perifocal zone demonstrated the sex differences in the content of E2 and T, the level of which was much higher in the females, and in P4, the concentration of which was found to be higher, on the contrary, in the males. At the same time, as we have shown earlier, the standard growth of Guerin's carcinoma in euthyroid rats had no significant sex differences in tumor volumes and animal life spans [9;10].

What was the case with the animals of the main group that led to changes in the dynamics of the tumor growth against the background of induced hypothyroidism? Our study showed that, in general, in the females of the main group, a much lesser saturation of the tumor and the perifocal zone thereof with estradiol and testosterone could be noted, compared with the indicators of the reference group that may have inhibited the growth of neoplasm. On the contrary, in the males of the main group, the tumor and its perifocal zone were more saturated with sex steroids than in the animals of the reference group, that, against the background of a lack of thyroid hormones in the blood serum and elevated TSH, provoked by hypothyroidism, probably made it possible for the tumor not to slow down its growth.

Our study showed that the blood counts did not reflect the changes, which were characteristic of the tu-

mor tissue and its perifocal zone. In blood of the intact animals, naturally, the level of estradiol was recorded to be higher in the females, and the levels of testosterone and progesterone were found to be greater in the males. The growth of Guerin's carcinoma in the animals of both sexes had an impact on the levels of hormones in blood, and as a result in the females the level of E2 sharply decreased, but the concentrations of T and P4 increased, while in the males, against the background of a decrease in E2, T also decreased, but P4 did not change. As a result, in the animals of the reference group with the independent tumor growth, the differences in the content of sex steroids in blood were practically leveled. In the main group, in the animals of both sexes, a change in the level of sex steroids occurred in blood, resulting in an increase in the level of E2, an increase in P4, and only in the females an increase in the level of T was reported.

It is known that the balance of the steroid hormones determines the features of the immune system, the nervous activity and the behavior, and, as a result, can affect the tumor growth. The female body, due to its physiological changes in the phases of the cycle, the processes of pregnancy, childbirth and lactation, is adapted to greater fluctuations in the content of sex steroids than it is the case with the male body. That is, on the one hand, the female body is a more flexible and hormonally labile system, and the occurrence rate of dysfunctional disorders, especially of the thyroid gland, is greater in women than in men, and, on the other hand, perhaps this is what makes it easier to compensate for a number of changes in the hormonal balance [14, 15].

Thus, both hypothyroidism and the growth of Guerin's carcinoma changed the level of sex hormones in blood, and, as a result, their combined effect led to a shift in the steroid balance in the tumor and its surrounding tissue. As a consequence, in the females, the saturation of the above zones with estrogens, androgens, and progesterone decreased, which could promote retardation of the growth of the tumor, while in the males, on the contrary, the tumor volume increased, so that no inhibition of the growth of Guerin's carcinoma was observed, despite the insufficiency of thyroid hormones.

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