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Development of an experimental model of tumor growth under hypothyroidism

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Abstract

Aim. Our aim has been to develop an experimental model of the tumor growth against the background of hypothyroidism in rats of both genders in order to study possible influence made by hypothyroidism on progression of malignant tumors of various histological types.

Materials and methods. In our studies we have used 100 outbred albino rats of both genders, with an individual body mass of 150-180 g. The female rats (n=30) and the male rats (n=30) have received Mercazolil at a day dosage of 2,5 mg/100g of the body weight for 30 days. After hypothyroidism in the treated rodents had been confirmed, one group of them (15 females and 15 males) were subcutaneously inoculated with the Guerin's carcinoma cells, and another group (covering other 15 females and other 15 males) has been undergone to transplantation of the Sarcoma 45 cells. The reference group has included the rats of both genders with subcutaneously inoculated the Guerin's carcinoma cells (n=10 females and n=10 males) and Sarcoma 45 cells (n=10 females and n=10 males), but without reproduction of the hypothyroidism model. Upon expiration of one month, within the 3 day period, we have estimated with a radioisotope analysis (RIA) standard assay kits (Immunotech, Czekh Republic) the levels of the thyroid hormones in blood of the tested animals as follows: Triiodothyronine (T3) (pM/L), total Thyroxine (T4) (pM/L) and Thyroid-Stimulating Hormon (TSH) (μU/mL). The obtained data have been processed with Statistica 10.0.

Results. Upon the treatment with Mercazolil, we have found in the females a decrease by a factor of 7,3 in the total level of Thyroxine and an increase by a factor 1,6 in the TSH level ($p<0,05$), while in the males we have recorded a reduction by a factor of 2 in the total level of Thyroxine and an increase by a factor of 1,5 in the TSH level ($p<0,05$). In this case, the average sizes of the tumors in the female rats with Guerin's carcinoma and hypothyroidism have been found smaller than those found in the reference group as given below: upon expiration of 4 days they are 1,3 times smaller ($p<0,05$), upon expiration of 7 and 10 days the volumes have been found 1,4 times smaller ($p<0,05$); upon expiration of 14 days the volumes have been recorded to be 1,5 times less ($p<0,05$); upon expiration of 18 days they have been reported to be 1,3 times less ($p<0,05$), and upon expiration of 21 days they have been estimated to be 1,4 times less ($p<0,05$). As to the males with Guerin's carcinoma and hypothyroidism, the average sizes of their tumors as against the reference group data have been recorded to be smaller as follows: upon expiration of 4 days they are found 13,3 times less; upon expiration of 7 days they have been recorded to be 7,5 times smaller; upon expiration of 10 days the volumes have been estimated to be 1,9 times less ($p<0,05$), and upon expiration of 14 days they have been found to be 2,6 times less. The survival rate in the female rats in the main test has been recorded to be 1,6 times higher ($p<0,05$) against the data in the reference group, while the survival rate in the males has not shown any significant differences therein. In the female rates with S 45 growing against the background of hypothyroidism the average sizes of the tumors have been found to be less than those identified in the reference group as follows: after 4 days, the sizes have been recorded to be 1,4 times less ($p<0,05$); after 7 and 10 days they have been recorded to be 1,6 and 3,2 times smaller, respectively ($p<0,05$); after 14 days they have been found to be 3,9 times less, and after 18 days they have been recorded to be 4,8 times less. In the males at tumor growth week stage 1, the tumor sizes have increased 3,1 times as against 4 days of the tumor growth; upon expiration of 10 days the sizes have been found to be 7,1 times greater as compared with the previous period; upon expiration of 2 weeks they have increased 1,5 times ($p<0,05$); upon expiration of 18 and 21 days the tumor sizes have been recorded to be greater by a factor of 2,3 and by a factor of 1,6, respectively ($p<0,05$). The life spans in the female rodents in the main test group has been reported to be longer by a factor of 1,8 ($p<0,05$) than it has been the case with the reference group, and the average life span in the males has reached 21 days.

Conclusion. We have revealed that in the female rates diagnosed with hypothyroidism the sizes of the subcutaneous tumor nodes of Guerin's carcinoma and S 45 show slower progression as against that recorded in the reference group, and

the life span recorded in the above rodents has been found as significantly longer, while in the male rats with hypothyroidism we have observed an irregular, slower, progression of the tumor nodes of Guerin's carcinoma and S 45 within the period of 14 days, but subsequently we have detected the same progression rate as it is the case with the reference group data.

Keywords

Hypothyroidism, Experimental model, Rats, Guerin's carcinoma, Thyroxine, Triiodothyronine, Thyroid-Stimulating Hormon

Imprint

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Introduction

The dynamic capability of biological systems to maintain the required flexible homeostatic balance by responding to environmental challenges is a salient feature of the normal health state in an organism. In this context, hormones released by the thyroid gland (thyroid hormones referred to as TH) are double-play actors in the homestatic regulation: first they are participators therein, and second they are at the same time controllable units. They are targeted at a wide range of metabolism effects, however, to a greater extent, at the same time they are externally controllable agents. Dysfunction of the thyroid gland including hypothyroidism is found in females more often as compared with males: the incidence rate is reported to be 2-9 times higher [1], but unfortunately in addition to the fertility and bone tissue aspects there is no proper understanding of what is possible influence made by the gender aspects on the typical features of various diseases: that's applicable to international level research and refers both to clinical studies and epidemiologic cohorts. At present, the generally accepted experimental approaches with the use of mice as models to investigate actions and effects produced by thyroid hormones have involved the experimental use of mice of the same gender only; moreover sometimes it is even ignored which gender is subjected to research studies. In 2014 Nature has appealed to the scientific community to make stronger efforts in exploring specific influences made by the gender on manifestation, prediction of possible outcome and treatment of diseases that subsequently has been supported by the Endocrine Society [2]. Besides "American thyroid association guide to investigating thyroid hormone economy and action in rodent and cell models" published in 2013 has issued recommendations to conduct gender-specific studies, since responding to TH disorders may differ depending on the gender [3].

Based on the mouse model, H. Rakov et al. have detected some apparent gender-related differences in the functional behavior, metabolic and biochemical parameters in mice diagnosed with hypothyroidism [4]. The above researchers have shown that it is precisely the gender that is an important modifier of actions and effects of the thyroid hormones that result in different phenotype-, metabolism- and biochemistry-related markers of hypothyroidism in male with female mice.

Experimental evidence data have confirmed the fact that Thyroxine (T4) and Triiodothyronine (T3) make their proliferative and an anti-apoptotic action and effects on cancer cells by regulating gene expression and stimulating estrogen-like effects that indicates that there is a relationship between the dysfunction of the thyroid gland and the risk of the onset of cancer [5].

However the relevant epidemiologic studies have supplied us with some contradictory data [6]. In a meta-analysis of some observation data published before 2019 there has not been revealed any statistically significant relation between hypothyroidism and a risk of the onset of breast cancer, but at the same time two recent research studies have declared that there is a lowering of risk of the onset of breast cancer linked with hypothyroidism [7, 8]. In contrast, some other investigations [9] have demonstrated that there is a higher risk of breast cancer in females with hyperthyreosis as compared with females having no dysfunction of the thyroid gland that has been supported by the results from another meta-analysis and a randomized study [7]. Following this way, revealed has been a more aggressive impact of the brain metastases under primary lung, breast and kidney cancer as well as skin melanoma on the hormonal status in the affected patients.

The authors thereof are of the opinion that the paraneoplastic abnormalities in the content of the thyroid hormones and cortisol should be taken into consideration in treatment of this sort of patients.

In view of the existing clinical context, when not only women, but also men suffer from hypothyroidism, taking into account the fact that the consequences of this disease, among them cancer, may be gender-dependent, it is reasonable to apply an experimental model in mice to provide an extensive, a more comprehensive, investigation of a possible influence of the gender on the actions and effects made by the thyroid hormones.

Development of experimental models is an integral part of research to discover the nature of malignant tumors and search for new ways to produce the desired effect thereon [10, 11, 12, 13, 14]. In this connection, the models confirming the substantial contribution of comorbidity to the onset and progression of malignant tumors are of particular interest. So, METHOD OF THE MELANOMA B 16 MALIGNANT GROWTH IN MICE MODIFICATION WITH CHRONIC PAIN (Pat. RU No. 2650587 C1), METHOD OF STIMULATING CHRONIC PAIN OF MALIGNANT GROWTH IN LUNG RATS (Pat. RU No. 2676641 C) and METHOD FOR REVERSING GENETICALLY DETERMINED INHIBITION OF GROWTH OF A MALIGNANT TUMOR IN EXPERIMENT (Pat. No.2718671 C1) [15,16,17] have shown that the chronic neurogenic pain is a factor stimulating the growth and progression of malignant tumors of different histogenesis, and it is even capable of reversing the genetically determined inhibition of the melanoma growth in animals with the urokinase gene knockout that implies an inhibition of its growth.

The aim of our experimental research has been a development of an experimental model of a tumor growth against the background of hypothyroidism in rats of both genders in order to investigate an influence made by hypothyroidism on progression of malignant tumors of different histological structures.

Materials and methods

Our experiment has been conducted using 100 outbred albino rats of both genders, with an individual body mass of 150–180 g. The experimental rodents have been supplied by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies" (Branch Andreevka, Moscow Region) at the Federal Medical & Biological Agency. The laboratory animals were kept under natural lighting conditions with free access to water and food. The research in the animals was completed in full compliance with the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, 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 the Russian Federation. Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antisepsis.

We have used in our research cell lines of Guerin's carcinoma and Sarcoma 45 delivered by the Federal State Budgetary Institution "N. N. Blokhin Russian National Oncology Research Center" at the Ministry of Health of the Russian Federation. The material for the inoculation was harvested from donor rats on tumor development day 12–16. The above mentioned laboratory animals were subcutaneously inoculated with the Guerin's carcinoma and Sarcoma 45 (S 45) cells by the standard subcutaneous injection of a volume of 0,5 ml of the tumor suspension cells in saline solution with a dilution ratio 1:10 in the right scapular region.

The outbred albino female rats (n=30) and male rats (n=30) have received Mercazolil at a day dose of 2,5 mg/100 g of body mass (the total dose is 75 mg/100 g of body mass) for 30 days. No avoidance of eating has been noted in the experiment with the animals; they have gained in their individual weight, but at the same time recorded have been deterioration of their external skin and hair coat appearance, some mobility problems and sleepiness in them. Upon obtaining the state of stable hypothyroidism with the use of Mercazolil for the purpose of investigations of an impact of the disease on the growth of malignant tumors of different histological structures, the animals of both genders (30 males and 30 females) with their confirmed hypothyroidism diagnosis have been divided into two test groups: one test group (15 females and 15 males) has been inoculated subcutaneously with the Guerin's carcinoma cells, and another test group (15 females and 15 males) has been inoculated subcutaneously with the Sarcoma 45 cells. For the purpose of tracing the hypothyroidism-free progression of the malignant tumor in the animals, we have used

the reference cohort of the rats of both genders, who had reached their sexual maturity and who had not be subjected to hyperthyroidism reproduction, to cover one group (n=10 females and n=10 males) inoculated subcutaneously with the Guerin's carcinoma cells and another (n=10 females and n=10 males) transplanted with the Sarcoma 45 line cells, at the same dose and with the same volume of the cell lines as it is the case with the main test group. Upon expiration of one month, within the 3 day period, we have estimated with a radioisotope analysis standard assay kits (Immunotech, Czekh Republic) the levels of Triiodothyronine (T3) (pM/L), total Thyroxine (T4) (pM/L) and Thyroid-Stimulating Hormon (TSH) (μU/mL).

The obtained experimental data have been processed with Statistica 10.0. The data have been analyzed for their compliance with the normal distribution law using the Shapiro-Wilk test (for small sample sizes). To make the comparison of more than two independent groups (for independent samples) we have utilized the Kruskal-Wallis test (when an independent variable consists of two or more categorical, independent groups). The table data are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean; in this case, $p<0.05$ has been taken as the level of statistical significance. The obtained results have been statistically processed in accordance with general guidelines applicable to medical research studies.

Results

Upon treatment of the female rats with Mercazolil for 30 days per os, we have found in their blood a decrease in the total level of Thyroxine by a factor of 7,3 and an increase in the TSH level by a factor of 1,6 ($p<0,05$), while in the males, who have been administered with Mercazolil for 30 days, a reduction 2 times in the total level of Thyroxine has been recorded and an increase 1,5 times in the TSH level has been reported ($p<0,05$) (see Table 1 herein). The detected low levels of Thyroxine and high levels of TSH after 30-day administration of Mercazolil at a dose of 2,5 mg/100 g of body weight bear witness to the fact that the designed stable state of hypothyroidism has been achieved.

Some specific features of the tumor growth upon the standard Guerin's carcinoma inoculation and those upon the same type inoculation against the background of hypothyroidism in rats of both genders are presented in Tables 2 and 3 herein.

Table 1

Level of thyroid hormones in serum in rats of both genders treated with Mercazolil

Groups of animals Hormones	T4 (pM/L)	T3 (pM/L)	TSH (μU/mL)
Intact females (n=10)	61,2±5,9	1,05±0,1	0,085±0,007
Mercazolil-treated females (n=30)	8,41±0,81[1]	1,25±0,11	0,14±0,013[1]
Intact males (n=10)	75,58±7,2	1,46±0,12	0,08±0,06
Mercazolil-treated males (n=30)	38,06±3,4[1]	1,11±0,09	0,12±0,009[1]

Note: [1] significant differences as compared with the respective values in the intact animals of the same gender $p<0,05$.

The subcutaneous tumor (Guerin's carcinoma) in the females in the main test group (n=15) has become detectable 4 days after the inoculation, and its average volume has reached at that time 0,125 cm^3. Upon the inoculation, the above tumor has been produced in 80% of the females, while in 20% (n=3) of the female rats with hypothyroidism no Guerin's carcinoma has developed. At week 1 stage of the tumor progression we have recorded an increase 18,2 times in the tumor mass as against the mass recorded on tumor growth day 4; on 10 day we have found an increase 5,8 times in the volume compared with the previous stage, and upon expiration of 2 weeks the volume has been recorded to be greater by a factor of 2,1. 3 weeks after the Guerin's carcinoma inoculation we have observed an inhibition of the tumor growth, so that the volumes of the tumor in the females in the main test group have been found to be 1,4 times ($p<0,05$) greater than those recorded after 18 days. Since day 11, with the tumor node enlargement in the animals, we have revealed some alopecia spots on the skin surface, which have shown a tendency towards their growth.

Upon expiration of 24 days of the tumor growth in the females in the main test group reported has been the first death case. Their average life span has been recorded to reach 29 days, with its maximum of 33 days (see Table 2 herein).

As to the females rats in the reference group (n=10), with the growing Guerin's carcinoma tumor against the normal levels of the thyroid hormones and TSH, the subcutaneous tumor was detectable after 4 days; its average volume has reached 0,165 cm^3; the tumor cell productivity has achieved 100%. One week after the inoculation the average volume of the tumor

Table 2

Tumor growth dynamics und survival data in female rats with Guerin's carcinoma

Time of testing	Main test group Hypothyriodism + Guerin's carcinoma (averaged tumor V cm^3)	Reference group Guerin's carcinoma only (tumor V cm^3)
4 days	0,125±0,012[1]	0,165±0,058
7 days	2,275±0,53[1]	3,18±0,33
10 days	13,21±0,926[1]	18,4±2,42
14 days	27,28±1,62[1]	44,76±3,98
18 days	55,94±5,4[1]	70,3±4,78
21 days	75,73±6,88[1]	107,96±9,01
Appearance of alopecia on skin surface	Beginning with day 11	Not available
Appearance of skin necrosis	Not available	Beginning with day 14
Average life span (days)	29,3± 1,16[1]	18,2±1,38
The first death case recorded in the group	On day 24	On day 13
The last death case recorded in the group	On day 33	On day 26

Note: [1] significant differences as compared with the respective values in the animals of the reference group p<0,05.

has increased by a factor of 1,9 (p<0,05) as compared with the data recorded after 4 days of the growth; the tumor volume increase by a factor of 5,8 has been identified upon expiration of 10 days, and upon expiration of 2 weeks it has been recorded to be increased 2,4 times as compared with the previous stages. Upon expiration of 18 days of the experiment the average volume of the tumor has been reported to be 1,3 times greater (p<0,05), and 3 weeks after the inoculation it has become 1,9 greater (p<0,05) as against the previous measuring stages. Beginning with week two, in the female rats in the reference group detected have been tumor necrosis cases. The first death case in the reference group has been reported on day 13, and the last case thereof has been recorded on day 26 of the experiment; the average life span of the animals has reached 18 days.

It has been revealed that in the females with the Guerin's carcinoma tumor growing against the background of hypothyroidism the average volumes recorded at all measuring stages are smaller than those found in the reference group as indicated below: on 4 day smaller by a factor of 1,3 (p<0,05), on day 7 and 10 it has decreased by a factor of 1,4 (p<0,05); upon expiration of 14 days it has been found 1,5 times smaller (p<0,05); upon expiration of 18 days it has been recorded 1,3 times smaller (p<0,05), and on day 21 it has become 1,4 times smaller (p<0,05). In this case the survival of the female rats in the main test group has been found to be 1,6 times higher (p<0,05) than it is the case with the rats in the reference group. The first death case in the main test group has been reported on day 24, i.e. 14 days later than it is the case with the animals in the reference group.

Our survey of the data dynamics of the tumor growth of inoculated Guerin's carcinoma against the background of hypothyroidism is presented in Table 3 given herein.

Table 3

Tumor growth dynamics und survival data in male rats with Guerin's carcinoma against the background of hypothyroidism

Time of testing	Main test group Hypothyriodism + Guerin's carcinoma (tumor V cm^3)	Reference group Guerin's carcinoma only (tumor V cm^3)
4 days	0,0375±0,0037[1]	0,5±0,035
7 days	0,5±0,0023[1]	3,82±0,27
10 days	7,94±0,80[1]	14,74±1,15
14 days	15,61±1,395[1]	40,68±3,8
18 days	44,9±3,74	52,84±5,48
21 days	72,93±7,09	77,5±6,25
Appearance of skin necrosis	Not available	Beginning with day 7
Average life span (days)	23,7± 2,12	20,0±1,27
The first death case recorded in the group	On day 20	On day 14
The last death case recorded in the group	On day 25	On day 24

Note: [1] significant differences as compared with the respective values in the animals of the reference group p<0,05.

The subcutaneous tumor (Guerin's carcinoma) in the male rats in the main test group (n=15) has become palpable 4 days after the inoculation, and its average volume has reached at that time 0,0375 cm^3. The capacity of the inoculated tumor cells to grow has been found in 100% of the males. At week 1 stage of the tumor growth, the tumor volume has increased by a factor of 13,3 as compared with the growth day 4 volume; after 10 days the volume has become larger by a factor of 15,9 against the data obtained in the previous test period; upon expiration of 2 weeks the volume has been recorded to be greater by a factor of 1,96 (p<0,05), and upon expiration of 18 days it has been detected to

be larger by a factor of 2,9. Later, 3 weeks upon the inoculation, the tumor volumes in the males in the main test group have increased 1,6 times ($p<0,05$) as compared with the respective data on day 18. The first death case in the main test group has been recorded on day 20 of the experiment. The average life span has reached in this case 23,7 days with its maximum of 25 days (see Table 3 herein).

As to the male rats in the reference group (n=10), their subcutaneous tumor has become detectable on day 4 after the inoculation with its volume of 0,5 cm^3; upon expiration of 7 days after the inoculation its average volume has become larger by a factor of 7,6; upon expiration of 10 days thereafter the tumor volume has increased 3,9 times and 14 days thereafter 2,8 times as against the measurements taken before. 18 days after the inoculation we have observed an inhibition of the tumor growth with an increase by a factor of 1,3 in its volume ($p<0,05$), and upon expiration of 21 days the volume has been reported to be larger by a factor of 1,5 ($p<0,05$). The first death case in the reference group has been recorded on day 14 of the Guerin's carcinoma inoculation experiment, and the last death case has been observed therein on day 29, so that the average life span in this group has been reported to be 27 days.

Considering the male rats in the main test group with growing Guerin's carcinoma against the background of hypothyroidism, as compared with the data obtained in the reference animals, the average volumes of the tumor at experiment stages from day 4 till day 14 have been found smaller as follows: on day 4 smaller by a factor of 13,3, on day 7 smaller by a factor of 7,5, on day 10 smaller by a factor of 1,9 ($p<0,05$), and on day 14 smaller by a factor of 2,6. However on day 18 and 21 we have not observed any significant differences therein from the data recorded in the males in the reference group.

Our survey of the experimental data obtained by us on the tumor volumes and life spans in the female rats with inoculated Sarcoma 45 against the background of hypothyroidism is given in Table 4 herein.

The subcutaneous tumor of S 45 in the main test group female rats (n=15) has become palpable on day 4 after the tumor cell transplantation, and its average volume has reached 0,29 cm^3. The experimental tumor in the female rats has been produced in 100% of the inoculation cases. At the week 1 stage of the tumor growth, the volume of the tumor has become larger by

Table 4

Tumor growth dynamics und survival data in female rats with inoculated Sarcoma 45 (S 45)

Time of testing	Main test group Hypothyriodism + S 45 (tumor V cm^3)	Reference group S 45 (tumor V cm^3)
4 days	0,29±0,012	0,23±0,020
7 days	0,68±0,06[1]	1,1±0,10
10 days	0,95±0,09[1]	3,08±0,31
14 days	1,39±0,14[1]	5,42±0,50
18 days	1,4±0,15[1]	6,68±0,7
Average life span (days)	32,4±1,2[1]	18,0±1,38
The first death case recorded in the group	On day 24	On day 12
The last death case recorded in the group	On day 34	On day 20

Note: [1] significant differences as compared with the respective values in the animals of the reference group $p<0,05$.

a factor of 2,3 as against its 4 day growth; on day 10 we have observed an increase by a factor of 1,4 in the tumor volume ($p<0,05$), as compared with the sizes recorded before; upon expiration of 2 weeks the volume has become larger by a factor of 1,4 ($p<0,05$).

Upon expiration of 18 days after the S 34 inoculation, we have observed an inhibition of the tumor growth, and the recorded sizes thereof have shown no differences therein from those measured before. On day 24 of the experimental tumor growth period in the main test group females we have observed the first death case, and the average life span has been reported to be 32 days with its maximum of 33 days (see Table 4 herein).

As to the examined female rats in the reference group (n=10) with growing S 45 against the background of the normal values of thyroid hormones, the tumor has been palpable underneath the skin on day 4 with the tumor node average volume of 0,23 cm^3, and in this group the tumor has been produced in 100% of the inoculation cases. Upon expiration of 1 week after the inoculation, the average volume of the tumor has increased 4,8 times as against the measurements taken on day 4; upon expiration of 10 days it has been recorded to be 2,8 times larger and after 2 weeks it has been observed to be 1,8 greater as compared with the respective previous experiment periods of time ($p<0,05$).

On day 18 of our experiment the average sizes of the growing tumor has been reported to be increased by a factor of 1, 3 ($p<0,05$). The first case of the death

of the animal in the reference group has been recorded on day 12, and the last case of the death has been reported on day 20, so that the average life span has reached 18 days.

We have revealed that the average volumes of the tumor in the females with S 45 growing against the background of hypothyroidism, measured at all stages of the experimental tumor progression, are smaller as compared with those found in the reference group animals: on day 4 the volume has been recorded to be smaller by a factor of 1,4 ($p<0,05$), on day 7 and day 10 smaller by a factor of 1,6 ($p<0,05$) and by a factor of 3,2, respectively; upon expiration of 14 days of the experiment it has decreased 3,9 times, and after 18 days of the experiment a reduction by a factor of 4,8 has been observed.

In this case, the survival for the female rats in the main test group has been reported to be 1,8 higher ($p<0,05$) than it is the case with that in the reference group rats. The first death case in the main group with S 45 has been recorded 12 days later as compared with the respective data obtained in the reference group.

The dynamics of progression of the subcutaneous S 45 tumor against the background of hypothyroidism in the male rats and their average life span data are indicated in Table 5 herein.

Table 5

Tumor growth dynamics und survival data in male rats with inoculated Sarcoma 45 (S 45)

Time of testing	Main test group Hypothyriodism + S 45 (tumor V cm^3)	Reference group S 45 (tumor V cm^3)
4 days	0,125±0,018	0,25±0,020
7 days	0,39±0,18	0,56±0,08
10 days	2,75±0,40	3,36±0,58
14 days	4,05±0,60	4,2±0,7
18 days	9,44±0,99	9,24±0,7
21 days	14,8±1,5	11,8±1,5
Appearance of skin necrosis	Not available	Beginning with day 14
Average life span (days)	21,3± 1,21	20,0±1,38
The first death case recorded in the group	On day 15	On day 14
The last death case recorded in the group	On day 22	On day 22

Note: [1] significant differences as compared with the respective values in the animals of the reference group $p<0,05$.

The S 45 tumor located subcutaneously in the male rats in the main test group (n=15) has become identifiable on day 4 after the experimental transplantation thereof, and its average volume has reached 0,125 cm^3. The experimental tumor productivity has been produced in 100% of the inoculation cases in the males. At the week 1 stage the volume of the tumor has increased by a factor of 3,1 as against the measurements taken on day 4 of the experiment; on day 10 the volume thereof has become larger by a factor of 7,1 as compared with the respective previous stage; 2 weeks after the tumor transplantation the volume has been recorded to be greater by a factor of 1,5 ($p<0,05$); upon expiration of 18 days it has become larger by a factor of 2,3, and upon measuring on day 21 it has been found to be greater by a factor of 1,6 ($p<0,05$). The average life time has been recorded to be 21 days with its maximum of 22 days (see Table 5 herein). Considering the male rats in the reference group (n=10) with growing S 45 against the background of the normal values of thyroid hormones including TSH, the experimental subcutaneous tumor has become palpable on day 4 of the experiment, and the tumor node volume has reached 0,25 cm^3; the experimental tumor inoculable capacity has achieved 100%. 1 week after the inoculation, the average volume of the experimental tumor has become 2,2 times larger as compared with that recorded on day 4; upon expiration of 10 days of the experiment it has been reported to be 6 times greater, and after 2 weeks the sizes thereof have been found to be larger by a factor of 1,25 ($p<0,05$) as against the data obtained in the respective previous period. On day 18 the average volume of the tumor has increased 2,2 times, and upon expiration of 3 weeks it has become greater by a factor of 1,3 ($p<0,05$). The first death case in this reference group has been observed on day 14, and the last death has been reported on day 22, so that the average life time in the animals has reached 20 days. As to the male rats in the main test group with growing S 45 against the background of hypothyroidism, it has been revealed that the average volumes measured within the period from day 4 till day 7 are smaller as listed below: on day 4 smaller by a factor of 2 and on day 7 by a factor of 1,4 ($p<0,05$), respectively. Upon analyzing the volume data related to test stages 10–21 days of growing S 45 no significant differences in the tumor volumes between the main test group males and the reference male rats have been detected. The average life spans in the males in the main test group have shown no differences from the values recorded in the males of the reference group.

Conclusion

Thus we may conclude that in the outbread albino female rats diagnosed with hypothyroidism (according to evidenced low values of total Thyroxine (T4) and high values of Thyroid-Stimulating Hormone (TSH) in serum) we have observed a delayed increase in the volumes of the tumor nodes produced by Guerin's carcinoma and S 45 upon their subcutaneous inoculation as compared with the respective progression tumor data observed in the reference group, and the life span has been recorded in them to be significantly longer. As to the outbread albino male rats with confirmed hypothyroidism (according to the confirmed low values of total Thyroxine (T4) and high values of Thyroid-Stimulating Hormone (TSH) in serum), we have found that the volumes of the tumor nodes, developed upon the subcutaneous Guerin's carcinoma and S 45 inoculation, have demonstrated an irregular pattern of their growth with some progression slow-down within the first 14 days of the experiment, followed by no-difference effects in the volume increase dynamics between the test and reference group. In this case, no significant differences in the life span values have been revealed. We think these effects should be attributed to a deficiency of Thyroxine as the main thyroid hormone responsible for many aspects in tumorogenesis. The pronounced effect recorded in the female rats in our study is determined by the predominance of estrogens as participants in the control and regulation in the thyroid gland performance.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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