

THE STUDY OF MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE THYROID FOLLICLES OF HEALTHY RATS AND RATS WITH EXPERIMENTALLY INDUCED HYPOTHYROIDISM FOLLOWING EXPOSURE TO MEDIUM-POWER LASER RADIATION

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Hypothyroidism remains a pressing concern. Laser irradiation is a widely used treatment option for patients with thyroid pathologies. Its efficacy depends on the applied dose. Changes in the form and volume of the structural components of the glands, such as thyrocytes and follicles, are dose-dependent and signal their functional state, which affects production, accumulation and secretion of thyroid hormones. The aim of our study was to explore the effect of infrared medium-power laser with total energy densities of 112 J/cm² and 450 J/cm² on the morphology and function of the thyroid and its follicles in health and hypothyroidism. The experiment was conducted in male rats. It was demonstrated that laser radiation affects the morphological state of thyrocytes and follicles of both intact animals and animals with experimentally induced hypothyroidism. Comparison of two laser regimens revealed that 112 J/cm² energies stimulated tissue regeneration and thyroid activity in general, whereas 450 J/cm² energies suppressed those processes. Our findings can be used to study hypothyroidism treatment options in the experimental setting.

Keywords: thyrocyte, follicle, nucleus/cell ratio, colloid accumulation index, laser, thyroid, hypothyroidism

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ИЗУЧЕНИЕ MORFOFУНКЦИОНАЛЬНЫХ ИЗМЕНЕНИЙ ФОЛЛИКУЛОВ ЩИТОВИДНОЙ ЖЕЛЕЗЫ КРЫС В НОРМЕ И ПРИ ГИПОТИРЕОЗЕ ПОСЛЕ ВОЗДЕЙСТВИЯ СРЕДНЕИНТЕНСИВНОГО ЛАЗЕРНОГО ИЗЛУЧЕНИЯ

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Проблема гипотиреоза в последнее время не теряет своей актуальности. При лечении тиреопатий успешно используется лазерное облучение щитовидной железы. Эффективность лазерного воздействия зависит от применяемой дозы излучения. Изменение формы и объема структурно-функциональных единиц органа (тиреоцитов и фолликулов) является дозозависимым процессом и отражает их функциональное состояние, влияющее на синтез, накопление и секрецию тиреоидных гормонов. Целью исследования было изучение влияния инфракрасного лазерного облучения средней интенсивности при суммарной плотности дозы с поверхности кожи 112 Дж/см² и 450 Дж/см² на морфофункциональное состояние тиреоцитов и фолликулов щитовидной железы в норме и при гипотиреозе. Эксперимент проведен на лабораторных крысах самцах. Показано, что лазерное воздействие изменяет состояние тиреоцитов и фолликулов как интактной щитовидной железы, так и при гипотиреозе. При сравнении эффектов двух изучаемых режимов лазерного воздействия на щитовидную железу с экспериментальным гипотиреозом повышение функции и регенеративных процессов железы отмечено при плотности дозы с поверхности кожи 112 Дж/см², и торможение при плотности дозы 450 Дж/см². Полученные результаты могут быть использованы для коррекции гипотиреоза в эксперименте.

Ключевые слова: тиреоцит, фолликул, ядерно-клеточное отношение, индекс накопления коллоида, лазерное излучение, щитовидная железа, гипотиреоз

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Hypothyroidism is an extremely common endocrine disorder. Because of the risk of complications affecting the normal function of other organs and systems and the lack of ideal treatment options, clinicians of different specialties take a serious interest in this condition [1]. There is an active ongoing search for novel therapies, some of which are laser-based. Here, priority is given to low-level laser therapy for the correction of

subclinical hypothyroidism [2, 3]. High-energy laser techniques ensuring a therapeutic effect in deep tissues are also underway [4, 5].

The thyroid is accessible to laser therapy as it lies close to the skin surface. Laser radiation can modulate its function, promote hormone secretion, improve microcirculation, and stimulate tissue regeneration. These laser effects are successfully

exploited in the treatment of hypothyroidism and autoimmune thyroiditis [6–10]. Some researchers believe that exposure to photons triggers structural changes in the thyroid stroma [10], affecting the height of the epithelium and the form and shape of follicles.

At present, the effect of different energy densities generated by a medium-power laser source on the functional activity of the animal thyroid remains understudied both in healthy animals and those with induced hypothyroidism.

The aim of this work was to study the effect of different energy densities of medium-power infrared laser radiation on the morphology and function of the thyroid epithelium and follicles in healthy animals and animals with induced experimental hypothyroidism.

METHODS

The study was conducted at the South Ural State Medical University and the Multidisciplinary Center for Laser Medicine, Chelyabinsk, between 2016 and 2018. We used 78 random-bred mature male rats weighing 200 to 220 g. The animals were kept in cages, 2–3 rats per cage, under standard day/night lighting conditions and fed a balanced diet *ad libitum*. Unlike females, male rats are not prone to hormone fluctuations and do not have estrus. Our study was conducted in compliance with animal welfare standards and guidelines, the *Rules for Carrying out Activities Involving Experimental Animals* (Addendum to Order No. 755 of the Ministry of Healthcare of the USSR dated September 12, 1977) and the Declaration of Helsinki (adopted in 1964 and revised in 1975, 1983 and 1989).

The animals were divided into 6 groups:

- 1) intact animals, no laser treatment applied;
- 2) intact animals; the total energy density applied to the thyroid was 112 J/cm² (0.5 W, 45 s);
- 3) intact animals; the total energy density applied to the thyroid was 450 J/cm² (1.5 W, 60 s);
- 4) animals with induced experimental hypothyroidism, no laser treatment applied;
- 5) animals with induced experimental hypothyroidism; the total energy density applied to the thyroid was 112 J/cm² (0.5 W, 45 s);
- 6) animals with induced experimental hypothyroidism; the total energy density applied to the thyroid was 450 J/cm² (1.5 W, 60 s);

Hypothyroidism was induced by daily oral gavage administration of 25 mg/kg 0.5 ml thiamazole in 0.9% isotonic sodium chloride solution prepared from Merkazolil (Akrikhin, Russia) *ex tempore*; the rats received the medication for 21 days [11]. The control group received 0.5 ml 0.9% NaCl per os for 21 days on a daily basis. Progression of hypothyroidism was assessed based on its clinical signs (changing body mass, appetite, fur appearance, and temperature), the morphological examination of the thyroid and the levels of thyroid hormones in blood serum.

The rats were irradiated with laser beams continuously for 5 days in a row, starting on day 22 of the experiment (a day after hypothyroidism induction was finished) using the IRE-Polus system (IRE-Polus, Russia).

The animals were anesthetized with ether and sacrificed by cervical dislocation on days 1, 7 and 30 following the completion of laser therapy (below referred to as days 1, 7 and 30).

Tissue samples were collected into 10% neutral buffered formalin solution for further histological analysis. Paraffin sections were prepared using a standard technique and then

stained with hematoxylin-eosin (pH 2.0). Microscopy was done at 400x magnification using the DMRXA microscope (Leica, Germany). The results were analyzed in ImageScope M, 2006 (Germany). We measured the height of the thyroid epithelium, the minimum and maximum follicular diameters and surface area. The height of the epithelium and follicular sizes were measured in 10 fields of view for each sample. To assess the functional activity of the thyroid, we used Braun's index (the index of colloid accumulation) calculated as the ratio of the inner follicular diameter to two heights of the follicular epithelium and the nucleus/cell ratio (the ratio of the nucleus area to the total cell area expressed in percent).

The data were analyzed in Microsoft Office Excel (2007) and SPSS Statistics 20 (2014) using non-parametric methods. The median, upper and lower quartiles were computed. To assess the significance of differences between the groups, we used the Mann-Whitney U test. Differences were considered significant at $p < 0.05$.

RESULTS

Histology revealed the intact lobular architecture of the thyroid in the controls. Connective tissue septa looked well defined. Tightly packed round and oval-shaped follicles were medium in size. The thyrocytes were cuboidal with distinct borders, constituting a layer of the follicular epithelium. The nuclei of the epithelial cells were round-shaped, lying at the base. Follicular lumens were evenly filled with purple-pink colloid, sometimes foamy along the edges (Fig. 1).

In the rats with induced hypothyroidism the thyroid retained its normal lobular structure. The stromal volume was increased. Connective tissue septa separating the lobes became looser, with areas of venous and capillary hyperemia and erythrocytosis. The follicles looked diminished in size; the colloid was light-colored or colorless. In some fields of view the thyrocytes appeared enlarged and had a pale foamy vacuolated cytoplasm. There were necrobiotic cells with pale nuclei; some of them totally lacked their nuclei (Fig. 2).

In the course of data analysis, we noticed that drug-induced hypothyroidism had led to certain changes in the stroma and parenchyma of the thyroid. Morphologically, the cells were expanded in volume as a result of severe hydropic and vacuolar degeneration. Their cytoplasm did not readily react with acid stains and looked pale pink, whereas in the intact animals the cytoplasm was homogenous, optically dense and readily reacted with acid stains. The underactive thyroid contained areas of severe degeneration and even necrobiosis or necrosis. The organ was enlarged, mainly due to the edema. The nucleus/cell ratio was low because of the expanded cytoplasm; changes in the nucleus area were not so pronounced.

Comparison of histologic samples between the groups revealed significant changes in the structural components of the thyroid (Table 1). The height of the thyroid epithelium shrank on days 1 and 7 but increased on day 30, whereas the minimum and maximum follicular diameters and follicular area diminished on days 7 and 30. The nucleus/cell ratio was low at all stages of the experiment, while Braun's index was increased on day 1 and decreased on day 30, as compared with the controls (Table 2).

Changes induced in the intact animals by the total energy dose of 112 J/cm² followed pretty much the same pattern throughout the experiment, except for the vascular response. Hyperemia and distended blood vessels were observed on a day following the start of treatment. The structure of the thyroid

was normal. Some follicles were enlarged, the colloid was bright pink and densely packed. The height of the epithelium was increased, whereas the diameter and area of the follicles were decreased in all experimental groups (Table 1). The nucleus/cell ratio went up, while Braun's index remained low at all stages of the experiment (Table 2).

Exposure to the total energy density of 450 J/cm² induced distension of the blood vessels and hyperemia in the thyroid of intact rats early in the experiment (on days 1 and 7). Upon irradiation the cytoplasm of the thyrocytes looked a bit swollen and finely granular. Some cells had a columnar shape; others were cuboidal. The colloid looked pink and fine-grained, the follicles were homogenous. On day 30 the gland structure of the irradiated rats was comparable to that of the controls, but the colloid still had a grainy texture.

The height of the thyroid epithelium was increased on days 1 and 30. Also, the minimum and maximum follicular diameters and their area expanded during the early stages of the experiment (days 1 and 7) and then gradually declined

reaching below the initial values by the end of day 30. Braun's index was significantly increased on day 7 and low on day 30.

In the animals with induced hypothyroidism irradiated with 112 J/cm² energies, the thyroid gland retained its lobular architecture; the stroma was well developed and the blood vessels were abundant (Fig. 3). The follicles were medium or large in size on day 1 and small on days 7 and 30. The follicular epithelium was cuboidal or prism-shaped, respectively, occasionally showing signs of proliferation and desquamation. The pale-blue colloid vacuolated along the edges was in intimate contact with the follicular wall. There were islands of the epithelium between the follicles. Although the epithelium height was increased throughout the experiment in the animals irradiated with 112 J/cm² energies, other studied parameters were low, including the minimum follicular diameter (throughout the experiment), the maximum follicular diameter (on day 1), and the area of the follicle on days 1 and 30. Braun's index was significantly decreased throughout the experiment, while the nucleus/cell ratio was increased on days 1 and 7.

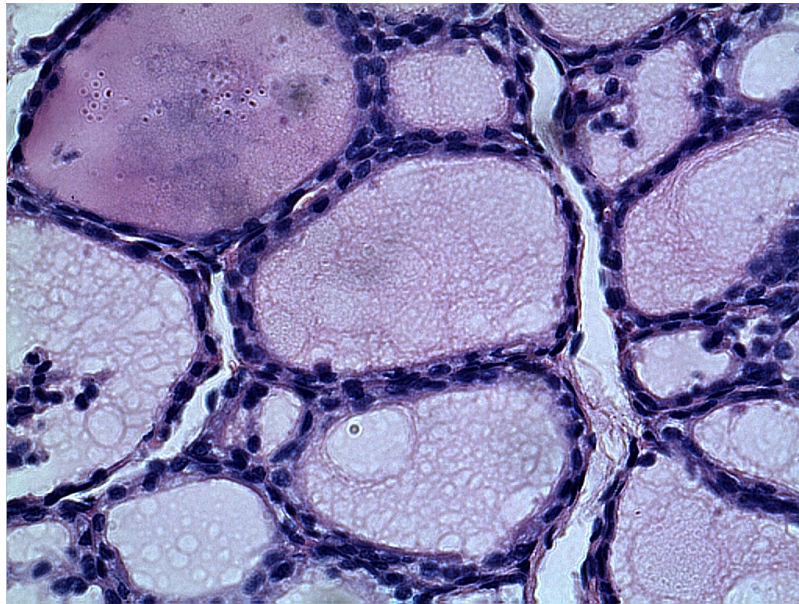


Fig. 1. The intact thyroid gland. Staining: hematoxylin-eosin; 400x magnification

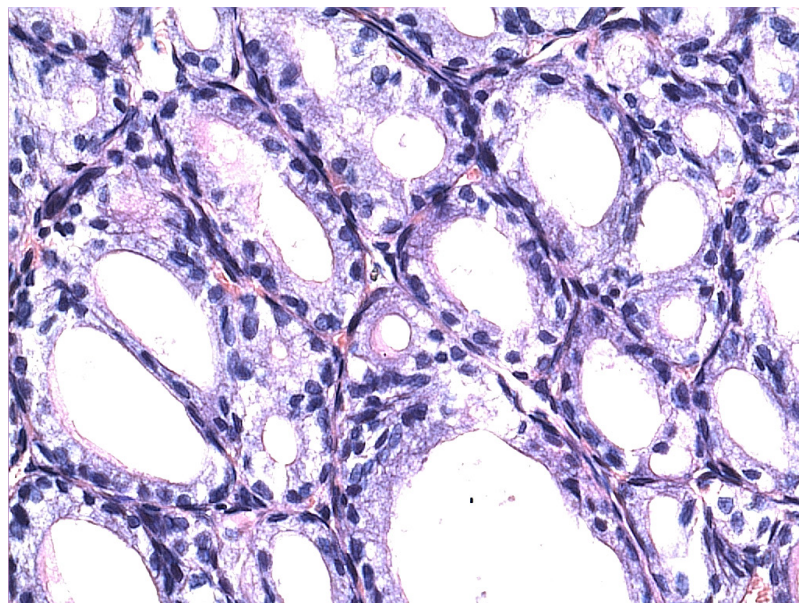


Fig. 2. Hypothyroidism. Staining: hematoxylin-eosin; 400x magnification

It the rats irradiated with 450 J/cm² energies, the stromal blood vessels looked pronouncedly distended on day 1. Erythrosthosis, red cell sludging and small hemorrhages per diapedesis were observed, the interstitium was moderately edematous. The follicles had a long irregular shape and "crinkled" walls. The thyrocytes were stricken by bad degeneration of protein constituents and developed necrobiosis or necrosis. Some groups of cells were desquamated into the follicular lumen. In some follicles the colloid accumulated along the follicle wall and was fine-grained; the lumens of other follicles were filled with layers of desquamated cells; there were a few almost empty lumens. On day 7 stromal venous

and capillary hyperemia and erythrosthosis were still present. Most of the follicles looked round-shaped; various degrees of dysproteinosis were observed in the thyrocytes (Fig. 4). In comparison with day 1, only a few follicles had desquamated cells inside. The colloid appeared as a pale-stained streak lying adjacent to the follicle wall in most follicles. On day 30 hyperemia was still present. The thyrocytes were cuboidal and showed signs of dysproteinosis of various degrees in some fields of view. The follicles had round or oval contours, the lumens were mostly empty. In some fields of view the follicles appeared to be filled with pale colloid and a few desquamated thyrocytes.

Table 1. Comparative analysis of the epithelium height, diameter and area of follicles in the experimental groups of animals

Groups		Epithelium height (µm)	Maximum diameter of the follicle (µm)	Minimum diameter of the follicle (µm)	Area of the follicle (µm ²)
Group 1: intact animals		5.41 (4.31; 6.06) ^{#1}	67.25 (51.83; 85.20) ^{#1}	39.85 (28.00; 65.23) ^{#1}	1936.55 (1162.15; 4469.65) ^{#1}
Group 2: intact animals. total energy density of 112 J/cm ²	Day 1	8.29 (7.49; 9.47) ^{#0}	53.30 (40.10; 71.13) ^{#0}	33.85 (26.58; 39.60) ^{#0}	1376.25 (847.14; 2150.53) ^{#0}
	Day 7	6.46 (6.05; 6.89) ^{#0}	41.15 (33.23; 56.53) ^{#0}	29.25 (22.40; 32.95) ^{#0}	947.22 (679.17; 1468.73) ^{#0}
	Day 30	6.25 (5.54; 6.94) ^{#0}	46.00 (33.03; 62.98) [#]	30.80 (25.60; 47.48) [#]	1123.71 (682.97; 2436.70) [#]
Group 3: intact animals. total energy density of 450 J/cm ²	Day 1	7.78 (6.74; 9.27) ^{#0}	144.00 (61.25; 232.00) ^{#0}	89.80 (46.05; 151.00) ^{#0}	10329.37 (2180.74; 31121.47) ^{#0}
	Day 7	5.12 (4.47; 5.75) ⁰	77.55 (60.03; 92.63) ⁰	50.70 (38.03; 61.38) ^{#0}	2917.37 (1823.94; 4287.75) ^{#0}
	Day 30	8.90 (7.82; 9.87) ^{#0}	49.15 (39.80; 56.05) [#]	30.15 (20.60; 36.10) [#]	1127.43 (658.19; 1591.63) [#]
Group 4: animals with induced hypothyroidism	Day 1	3.47 (3.03; 3.90) ^{1*}	65.25 (46.43; 88.98) [*]	45.05 (31.98; 56.28) [*]	2409.08 (1196.19; 3843.20) [*]
	Day 7	4.19 (3.84; 4.66) ^{1*}	47.75 (40.65; 54.38) ^{1*}	33.65 (27.53; 44.78) [*]	1182.59 (851.75; 1943.46) ^{1*}
	Day 30	5.87 (4.81; 6.85) ^{1*}	40.00 (31.15; 50.00) ^{1*}	24.45 (20.25; 29.73) ^{1*}	779.69 (506.25; 1086.82) ^{1*}
Group 5: animals with induced hypothyroidism. total energy density of 112 J/cm ²	Day 1	7.76 (6.81; 8.63) ^{#0}	40.50 (31.40; 52.83) ^{#0}	22.60 (16.28; 28.93) ^{#0}	734.71 (423.79; 1153.31) ^{#0}
	Day 7	11.00 (9.73; 12.40) ^{#0}	44.70 (33.83; 59.60) ⁰	27.10 (20.68; 31.20) ^{#0}	898.41 (623.40; 1390.14) ^{#0}
	Day 30	7.63 (7.00; 8.27) ^{#0}	37.75 (29.48; 44.35) ⁰	21.50 (18.93; 25.05) ^{#0}	656.24 (464.05; 865.92) ⁰
Group 6: animals with induced hypothyroidism. total energy density of 450 J/cm ²	Day 1	6.23 (5.43; 7.33) ^{#0}	207.00 (95.53; 344.00) ^{#0}	163.00 (58.35; 233.25) ^{#0}	28338.69 (4115.17; 61410.05) ^{#0}
	Day 7	4.45 (4.06; 4.96) ^{#0}	73.85 (49.08; 91.68) ^{0*}	42.70 (36.45; 61.58) ^{#0}	2504.98 (1466.21; 4167.30) ^{#0}
	Day 30	6.06 (5.25; 6.77) ⁰	47.55 (40.48; 59.48) ^{#0}	24.70 (22.10; 30.18) ⁰	979.59 (693.59; 1278.27) ^{#0}

Note: ¹p < 0.05, intact controls compared with animals with untreated induced hypothyroidism; [#]p < 0.05, irradiated animals compared with intact controls; *p < 0.05, irradiated animals compared with animals with untreated induced hypothyroidism; ⁰p < 0.05, comparison between the groups of irradiated animals.

Table 2. Comparative analysis of the nucleus/cell ratio and Braun's index in the experimental groups of animals

Groups		Nucleus/cell ratio	Braun's index
Group 1: intact animals		32.78 (29.25; 34.43) ^{#1}	4.76 (3.76; 6.72) ^{#1}
Group 2: intact animals, total energy density of 112 J/cm ²	Day 1	49.04 (42.99; 55.35) ^{#0}	2.95 (2.42; 3.46) ^{#0}
	Day 7	40.04 (32.39; 43.18)	2.94 (2.54; 3.56) ^{#0}
	Day 30	36.19 (32.07; 43.72) ⁰	4.37 (3.62; 4.97) ⁰
Group 3: intact animals, total energy density of 450 J/cm ²	Day 1	30.02 (25.54; 35.62) ⁰	3.50 (3.31; 6.79) ⁰
	Day 7	36.49 (26.44; 46.73)	5.97 (4.78; 7.02) ^{#0}
	Day 30	31.66 (25.76; 36.63) ⁰	2.49 (2.20; 2.89) ^{#0}
Group 4: animals with induced hypothyroidism	Day 1	14.90 (13.17; 21.21) ^{1*}	8.04 (5.74; 9.49) ^{1*}
	Day 7	23.67 (18.92; 26.45) ^{1*}	4.72 (4.06; 5.95) [*]
	Day 30	23.79 (20.75; 29.64) ¹	2.74 (2.21; 3.28) ^{1*}
Group 5: animals with induced hypothyroidism, total energy density of 112 J/cm ²	Day 1	29.82 (26.26; 36.28) [*]	2.46 (2.10; 2.90) ^{#0}
	Day 7	29.08 (27.24; 37.89) ^{#0}	1.71 (1.39; 2.05) ^{#0}
	Day 30	25.87 (22.42; 32.37)	2.06 (1.92; 2.39) ^{#0}
Group 6: animals with induced hypothyroidism, total energy density of 450 J/cm ²	Day 1	30.18 (22.63; 31.87) [*]	5.69 (4.64; 8.50) ⁰
	Day 7	25.51 (19.50; 29.03) ⁰	4.76 (4.23; 6.02) ⁰
	Day 30	27.33 (23.31; 33.10)	2.93 (2.57; 3.23) ⁰

Note: ¹p < 0.05, intact controls compared with animals with untreated induced hypothyroidism; [#]p < 0.05, irradiated animals compared with intact controls; *p < 0.05, irradiated animals compared with animals with untreated induced hypothyroidism; ⁰p < 0.05, comparison between the groups of irradiated animals.

DISCUSSION

The structural and functional unit of the thyroid is a follicle, a bubble with a cavity inside. Healthy rats have round or oval-shaped follicles evenly dispersed across the thyroid parenchyma. The follicular wall is lined with a single layer of the epithelium consisting of follicular thyrocytes. The cavity of the follicle is filled with colloid secreted by thyrocytes. The height of the epithelium, the shape and volume of the follicles change depending on the functional state of the thyroid. In healthy animals the thyrocytes have a cuboidal shape and colloid production and resorption are in equilibrium. In the underactive thyroid the epithelium becomes flat and the follicles enlarge in size. In the hyperactive thyroid the thyrocytes acquire a columnar shape and the follicular volume diminishes because of colloid resorption. The functional activity of the epithelium can be measured using the ratio of the nucleus area to the cell area (high values mean increased activity) and Braun's index (low values mean increased activity).

The lowered values of the nucleus/cell ratios, the shorter height of the thyroid epithelium and increased Braun's index, as well as the expanded area of the follicles observed on day 1 indicate a decline in the functional activity of the thyroid confirmed by thyroid hormone levels in the blood serum measured in our previous work [12]. On days 7 and 30 of the experiment the epithelium was gradually becoming taller, the nucleus/cell ratio was growing and the maximum and minimum follicular diameters, the area of the follicle, and Braun's index were decreasing, evident of thyroid recovery.

The changes occurring in the intact animals irradiated with a total energy dose of 112 J/cm² can be explained by the stimulating effect of laser beams that triggered a cascade of cellular pathways improving the microcirculation of the gland and promoting angiogenesis.

Laser photons are absorbed by membrane chromophores that have an appropriate absorption spectrum, modifying redox processes in the cell and affecting the permeability of calcium channels [13, 14].

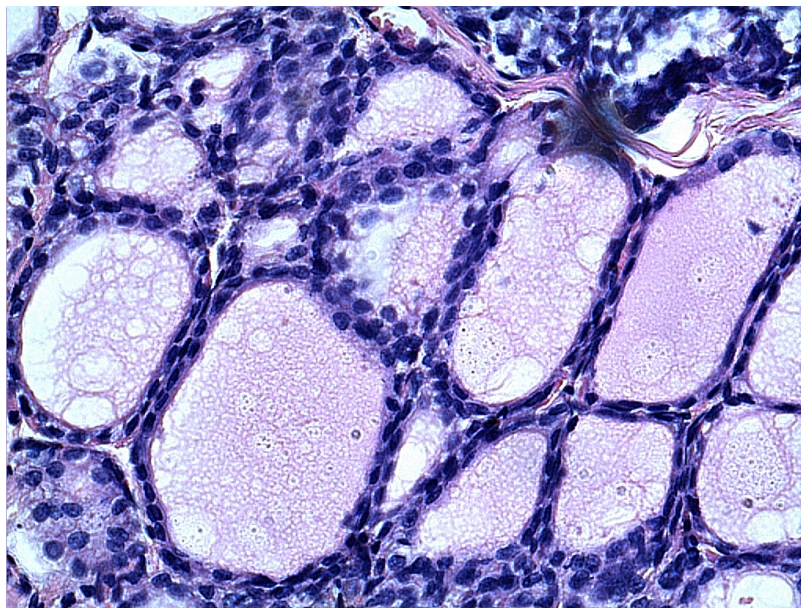


Fig. 3. The thyroid of experimental animals on day 7 after irradiation with 112 J/cm² laser beams. Staining: hematoxylin-eosin; 400x magnification

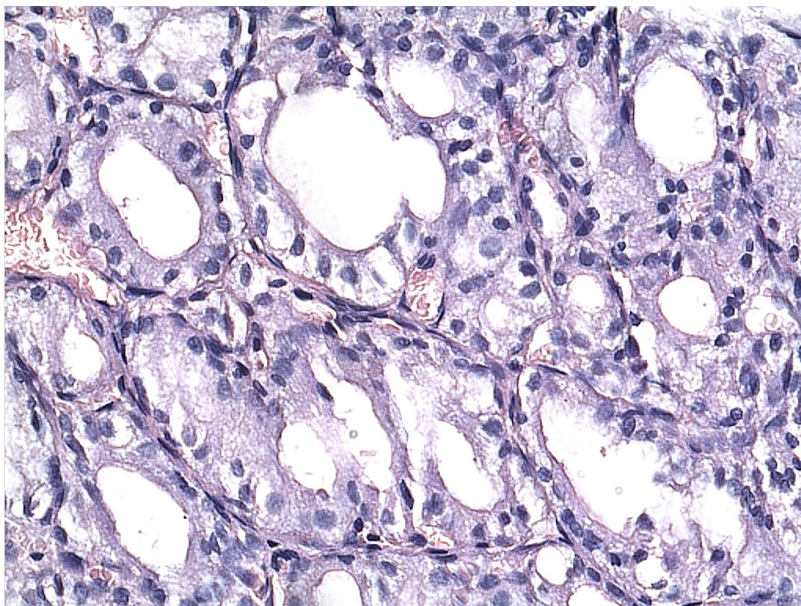


Fig. 4. The thyroid of experimental animals on day 7 after irradiation with 450 J/cm² laser beams. Staining: hematoxylin-eosin; 400x magnification

According to the literature, among the effects of laser radiation is stimulation of microcirculation in the thyroid associated with the local synthesis of nitric oxide [3, 6]. Improved microcirculation promotes synthesis of thyroid hormones by the thyroid epithelium [10, 15, 16].

In our study the changes induced in the thyroid of the intact animals by 450 J/cm² energies were very pronounced. This can be explained by the rough effect of laser radiation [17]. Possibly, the use of high energy densities triggered oxidative stress and induced irreversible changes in the membrane and structural proteins of thyrocytes [18].

Irradiation of the underactive thyroid of experimental animals with energy densities of 112 J/cm² led to an increase in the epithelium height and the nucleus/cell ratio and a decrease in the maximum and minimum diameter and area of the follicles. Braun's index also demonstrated a decline. All those changes were more pronounced during the early stages of our experiment on days 1 and 7. This suggests a stimulating effect of a 112 J/cm² dose on the functional activity of the underactive thyroid and characterizes processes of regeneration occurring in the thyroid damaged by an antithyroid agent. Our previous study [12] revealed reduced levels of the thyroid stimulating hormone (TSH) and increased levels of free and bound T4 and T3 in the underactive thyroid exposed to 112 J/cm² laser energies, which suggests a stimulating effect of laser radiation.

Morphological changes occurring in the thyroid following irradiation with 450 J/cm² energies during this study and the results of hormone titration measured in our previous work lead us to conclude that such irradiation downregulates the thyroid function.

The analysis of histological sections revealed an increase in all studied parameters, including statistically significant changes in the epithelium height and in the minimal follicular diameter during the early stages of the experiment (on days 1 and 7), whereas the maximum diameter of the follicle and its

area were increased throughout the experiment. A significant increase in the nucleus/cell ratio was observed 24 hours after irradiation.

Comparison of two irradiation regimens applied to the intact animals revealed that the epithelium was significantly taller during the early stages of the experiment (days 1 and 7) and shorter on day 30 following irradiation with 112 J/cm² energies. The maximum and minimum diameters of the follicle and the follicle area were decreased on days 1 and 7. Braun's index was low on days 1 and 7, rising by day 30. The cell/nucleus ratio was high on days 1 and 30.

Comparison of two irradiation regimens applied to the animals with experimentally induced hypothyroidism showed that a dose of 450 J/cm² induced a significant decrease in the epithelium height and an increase in other parameters observed throughout the experiment. Braun's index was also increased. The nucleus/cell ratio shrank considerably on day 7.

Our study demonstrates that laser radiation has a dose-dependent effect on the thyroid, stimulating its functional activity at energy densities of 112 J/cm² more pronounced during the early stages of the experiments (days 1 and 7) and inhibiting its activity at energy densities of 450 J/cm².

CONCLUSION

Medium-power laser radiation induces significant changes in the morphology and function of thyroid follicles in healthy rats and rats with induced hypothyroidism. The stimulating effect of infrared laser beams with a total energy density of 112 J/cm² on the underactive thyroid of male rats is more pronounced on days 1, whereas 450 J/cm² energies suppress the thyroid function throughout the whole follow-up period.

Our findings suggest that the use of infrared laser with a 112 J/cm² energy density on the skin surface is preferable when studying hypothyroidism treatment options in the experimental setting.

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