DYNAMICS OF FUNCTIONAL ACTIVITY OF THYROCYTES IN THE SETTING OF CHANGING MORPHOFUNCTIONAL ACTIVITY OF MAST CELLS OF THE THYROID GLAND UPON INFRARED LASER THERAPY

Smelova IV[1] ✉, Golovneva ES[2]

[1] Multi-specialty Center of Laser Medicine, Chelyabinsk, Russia

[2] South Ural State Medical University, Chelyabinsk, Russia

Exposure to laser radiation is an interesting method of treating hypothyroidism and autoimmune thyroiditis. Its effect depends on the applied radiation dose. Degranulation of stromal mast cells of the thyroid gland (mastocytes) is dose dependent; release of granular contents into the surrounding tissues may affect microcirculation, result in the increased activity of the thyroid epithelium or stimulate the thyroid. Our study aimed to investigate the effect of moderate-intensity infrared laser radiation at total surface doses of 112 and 450 J/cm^2 on the functional state of mast cells and blood serum levels of thyroid gland hormones in healthy and hypothyroid subjects. The experiment was carried out in 78 random-bred mature male rats. Hypothyroidism was modeled by *per os* administration of 25 mg/kg Mercazolil (Akrikhin, Russia) for 21 days. Radiation therapy was performed using a IRE-Polus laser with a wavelength of 970 nm (NTO IRE-Polus, Russia). The animals received radiation therapy for 5 days and were sacrificed 1, 7, and 30 days after the experiment. Subsequently, we calculated the total number of mast cells, degranulated mastocytes, mastocytes with degree 1, 2 and 3 degranulation, degranulation coefficient, and levels of TSH in blood serum. Mast cells of the intact thyroid gland demonstrated low sensitivity to laser radiation, but hormone levels changed soon after radiation was discontinued. In the animals with hypothyroidism induced by thiamazole (the active component of Mercazolil), activation of mast cells was observed in the recovery period after the drug was discontinued. Hypothyroidism was accompanied by changes in TSH, T_4 and T_3 levels in blood serum. Comparison of the effects of two laser therapy modes in the animals with induced hypothyroidism revealed increased functional activity of mastocytes and normalized levels of secreted hormones at a total dose of 112 J/cm^2 and reduced mast cell activity at a total dose of 450 J/cm^2.

Keywords: hypothyroidism, thyroid gland, mast cells, mastocytes, degranulation, thyroid stimulating hormone, laser radiation

✉ **Correspondence should be addressed:** Irina Smelova
ul. Potemkina 14, kv. 65, Chelyabinsk, Russia, 454081; spiral.siv@mail.ru

Received: 28.11.2016 **Accepted:** 12.12.2016

ДИНАМИКА ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ ТИРЕОЦИТОВ ПРИ ИЗМЕНЕНИИ МОРФОФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ТУЧНЫХ КЛЕТОК ЩИТОВИДНОЙ ЖЕЛЕЗЫ ПОД ВОЗДЕЙСТВИЕМ ИНФРАКРАСНОГО ЛАЗЕРНОГО ИЗЛУЧЕНИЯ

И. В. Смелова[1] ✉, Е. С. Головнева[2]

[1] Многопрофильный центр лазерной медицины, Челябинск

[2] Южно-Уральский государственный медицинский университет, Челябинск

Лазерное воздействие представляет интерес в качестве метода терапии гипотиреоза и аутоиммунных тиреоидитов. Его эффективность зависит от применяемой дозы излучения. Дегрануляция стромальных тучных клеток щитовидной железы (мастоцитов) является дозозависимым процессом, а попадание содержимого клеточных гранул в окружающие ткани может привести к изменению микроциркуляции, повышению активности тиреоидного эпителия и усилению эндокринной функции железы. Целью исследования являлось изучение влияния инфракрасного лазерного облучения средней интенсивности при суммарной плотности дозы с поверхности кожи 112 и 450 Дж/см^2 на функциональное состояние тучных клеток и содержание гормонов щитовидной железы в сыворотке крови в норме и при гипотиреозе. Эксперимент провели на 78 беспородных половозрелых самцах крысы. Гипотиреоз моделировали пероральным введением «Мерказолила» («Акрихин», Россия) в дозе 25 мг/кг в течение 21 дня. Облучение производили с использованием аппарата «ИРЭ-Полюс» с длиной волны 970 нм (НТО «ИРЭ-Полюс», Россия) ежедневно в течение 5 дней. Животных выводили из эксперимента на 1, 7 и 30 сутки. Подсчитывали общее количество тучных клеток, количество дегранулированных мастоцитов, количество мастоцитов I, II и III степени дегрануляции, коэффициент дегрануляции, а также содержание в сыворотке крови тиреотропных гормонов. Показано, что тучные клетки интактной щитовидной железы малочувствительны к лазерному воздействию, но гормональный профиль изменяется в ранние сроки после облучения. При моделировании гипотиреоза тиамазолом (действующее вещество «Мерказолила») происходит активация тучных клеток в восстановительном периоде после прекращения дачи препарата. Моделирование гипотиреоза сопровождается изменением содержания в сыворотке крови гормонов ТТГ, T_4 и T_3. При сравнении результатов воздействия двух режимов лазерного излучения на железу животных с экспериментальным гипотиреозом отмечается повышение функциональной активности мастоцитов и нормализация уровня гормональной секреции при суммарной плотности дозы 112 Дж/см^2 и снижение активности тучных клеток при суммарной плотности дозы 450 Дж/см^2.

Ключевые слова: гипотиреоз, щитовидная железа, тучные клетки, мастоциты, дегрануляция, тиреотропный гормон, лазерное излучение

✉ **Для корреспонденции:** Смелова Ирина Викторовна
ул. Потемкина, д. 14, кв. 65, г. Челябинск, 454081; spiral.siv@mail.ru

Статья получена: 28.11.2016 **Статья принята к печати:** 12.12.2016

Over the last decade, new laser-based technologies have become increasingly integrated into clinical routine. In addition to popular low-level lasers [1], high-power lasers are also employed by new treatment techniques to achieve therapeutic effects in deep tissues [2, 3].

Because the thyroid gland is a superficial organ, laser beams can easily reach it percutaneously. Laser radiation modulates thyroid function, stimulates secretion of thyroid hormones, improves microcirculation in the thyroid and produces a positive effect on tissue repair; it is widely used to treat hypothyroidism and autoimmune thyroiditis [4–7]. Some researchers associate structural changes in the thyroid epithelium with the effect of light photons on the thyroid stroma and its immune cells in particular. It is known that low-level laser irradiation of the thyroid stimulates secretion of thyroid hormones [8].

Thyroid hormones work by binding to the receptors in the cell nucleus; they regulate metabolism, control enzyme activity, and stimulate tissue growth, development and differentiation. Unquestionable is the effect of the thyroid hormones on the central and peripheral nervous systems, higher nervous activity and other endocrine glands.

The major function of the thyroid stimulating hormone (TSH) produced by the anterior pituitary is to exert a feedback control over synthesis and release of thyroid hormones. Thyroid function can be evaluated by thyroxine (T_4) and triiodothyronine (T_3) levels in blood serum, especially by measuring their unbound fractions which are a reliable indicator.

Stromal mast cells produce a variety of mediators, cytokines and enzymes, which determines the wide range of their activities. They were shown to participate in microcirculation and angiogenesis, tissue response to extreme conditions, inflammation and allergic reactions [9]. Bioactive substances released by degranulating mast cells affect the microvasculature and follicular epithelium, play an important role in the reparative regeneration of the thyroid and can change its functional activity [10]. Exposed to a laser, mast cells degranulate [11, 12].

The effect of varying doses of medium-level laser radiation on the functional activity of thyroid mast cells is still understudied. The aim of this work was to investigate the effect of different doses of medium-level infrared laser radiation on the functional activity of stromal mast cells by the assessment of their degranulation degree and on the functional activity of thyrocytes by the assessment of blood serum levels of thyroid hormones in healthy and hypothyroid subjects.

METHODS

The experiment was carried out in 78 random-bred adult male rats (body weight of 200–220 g) who were housed in cages, 2–3 animals per cage, ad libitum, under standard lighting conditions (day/night) and received a balanced diet. The experiment complied with 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 revisions of the years 1975, 1983 and 1989 of The Declaration of Helsinki (1964).

The animals were divided into 6 groups: 1) intact animals; 2) animals with induced hypothyroidism; 3) intact animals who received a total radiation dose of 112 J/cm^2 (exposure time of 45 s); 4) intact animals who received a total radiation dose of 450 J/cm^2 (exposure time of 60 s) 5) animals with induced hypothyroidism who received a total radiation dose of 112 J/cm^2 (exposure time of 45 s); 6) animals with induced hypothyroidism who received a total radiation dose of 450 J/cm^2 (exposure time of 60 s). The animals were irradiated 5 days in a row; irradiation started one day after the induction of hypothyroidism. We used the IRE-Polus laser with a wavelength of 970 nm (IRE-Polus, Society for Research and Technology, Russia). The chosen irradiation mode with a total dose of 450 J/cm^2 did not cause any thermal damage to thyroid tissues.

Hypothyroidism was induced by a 21-day oral administration of 25 mg/kg Mercazolil (thiamazole by Akrikhin, Russia) by gavage [13]. Intact animals received the same dose of 0.9 % sodium chloride per os. Hypothyroidism progression was assessed by its clinical signs: changing body weight, appetite, hair condition, and temperature, considering thyroid morphology and the levels of thyroid hormones in blood serum. The animals were anesthetized with ether and sacrificed by cervical dislocation 1, 7 and 30 days after laser treatment was over.

Tissue samples were placed in 10 % neutral buffered formalin, and standard paraffin sections were prepared. The sections were then stained with hematoxylin-eosin and toluidine blue (pH = 2.0). Microscopy was performed using the DMRXA microscope (Leica, Germany). Images were transmitted to and analyzed by the DiaMorph Cito-W software (Russia). The morphometric analysis determined the total number of mast cells and the number of mast cells at different degranulation stages; the value of the degranulation coefficient was calculated. Mast cells were counted in 10 fields of view in each paraffin section; their number was then expressed as cells per 1 mm^2. Mast cells were subgrouped depending on their degranulation degree: 1) 1st degree — 1–2 granules outside the cytoplasm; 2) 2nd degree — 3–10 granules outside the cytoplasm; 3) 3rd degree— over 10 granules outside the cytoplasm. The degranulation coefficient was calculated as a ratio of degranulated cells to the total number of mast cells.

The levels of thyroid hormones in blood serum were measured by enzyme immunoassay using the fully automated Personal LAB analyzer (Adaltis, Italy) and two reagent kits: one kit by Cusabio Biotech (China) was used to determine TSH levels, and the other by Vector-Best (Russia) was used to measure the levels of bound and unbound thyroxine and triiodothyronine.

Nonparametric statistical analysis was performed using Microsoft Excel and SPSS Statistics 20 (IBM, USA). The median, the upper and lower quartiles were calculated. To assess the significance of difference, Mann–Whitney U-test was used. Difference was considered significant at $p < 0.05$.

RESULTS

The total number of thyroid mast cells did not differ significantly in the untreated hypothyroid animals and the intact animals (Table 1). The number of mast cells of the 1st degree of degranulation was lower in the animals who were sacrificed 1 day after hypothyroidism induction was completed compared to the intact animals (Table 2). The same experimental groups demonstrated a statistically significant increase in the number of mast cells of the 2nd and 3rd degrees of degranulation and the increased value of the degranulation coefficient in the animals sacrificed 1 month after irradiation.

Unbound T_4 was significantly reduced and TSH was significantly elevated in the hypothyroid animals compared to the intact animals regardless of the time of sacrifice (Table 3).

No changes in the number of mast cells and their activity were observed in the thyroid of the irradiated intact animals sacrificed in the early stages of the experiment (24 hours and 7 days after irradiation) regardless of the total radiation dose

Table 1. Comparison of animal groups by the total number of mast cells, including degranulated cells, and the degranulation coefficient

Group		Total number of mast cells (per mm^2)	Number of degranulated mast cells (per mm^2)	Degranulation coefficient
Group 1: intact animals		15.4 (7.48;21.78)	5.06 (4.07;10.56)$^{\#,1}$	0.53 (0.35;0.58)$^{\#,1}$
Group 2: intact animals; a total radiation dose of 112 J/cm^2	24 hours after the experiment	8.58 (7.32;15.02)	4.07 (3.52;6.44)	0.45 (0.38;0.55)
	1 week after the experiment	10.78 (7.59;16.61)	6.27 (4.84;11.66)	0.66 (0.53;0.73)
	1 month after the experiment	18.15 (15.90;23.05)	12.87 (10.45;15.62)$^{\#,\circ}$	0.71 (0.59;0.80)$^{\#,\circ}$
Group 3: intact animals; a total radiation dose of 450 J/cm^2	24 hours after the experiment	11.65 (9.96;11.88)	3.30 (2.64;6.11)	0.40 (0.23;0.57)
	1 week after the experiment	13.20 (7.87;16.34)	3.96 (3.69;11.72)	0.46 (0.30;0.72)
	1 month after the experiment	12.54 (8.36;19.03)	4.84 (2.42;8.36)$^{\circ}$	0.36 (0.30;0.49)$^{\circ}$
Group 4: animals with induced hypothyroidism	24 hours after the experiment	13.86 (8.75;14.19)*	5.83 (5.34;6.82)*	0.47 (0.40;0.71)*
	1 week after the experiment	12.87 (8.47;16.23)	6.60 (5.17;11.61)	0.67 (0.39;0.79)
	1 month after the experiment	17.60 (16.28;20.68)*	14.74 (11.66;15.62)^{1,*}	0.84 (0.73;0.92)^{1,*}
Group 5: animals with induced hypothyroidism; a total radiation dose of 112 J/cm^2	24 hours after the experiment	18.81 (16.78;20.85)*	17.05 (15.24;18.87)$^{*,\circ}$	0.91 (0.90;0.91)$^{*,\circ}$
	1 week after the experiment	15.51 (13.59;18.59)*	12.21 (11.06;16.17)$^{\circ}$	0.82 (0.78;0.87)$^{\circ}$
	1 month after the experiment	12.32 (10.34;15.18)*	10.78 (9.57;12.87)$^{*,\circ}$	0.86 (0.83;0.95)$^{\circ}$
Group 6: animals with induced hypothyroidism; a total radiation dose of 450 J/cm^2	24 hours after the experiment	10.34 (8.58;14.41)*	6.27 (4.57;8.31)$^{\circ}$	0.55 (0.52;0.66)$^{\circ}$
	1 week after the experiment	9.35 (6.44;10.29)*	5.28 (3.80;6.60)$^{\circ}$	0.60 (0.55;0.65)$^{\circ}$
	1 month after the experiment	15.18 (14.63;16.06)	5.17 (4.57;5.94)$^{*,\circ}$	0.35 (0.29;0.40)$^{*,\circ}$

Notice. Data are presented as a median (lower quartile; upper quartile)

^{1} — $p < 0.05$ when comparing groups 4 and 1;
$^{\#}$ — $p < 0.05$ when comparing groups 2 and 3 with group 1;
* — $p < 0.05$ when comparing groups 5 and 6 with group 4;
° — $p < 0.05$ when comparing group 2 with group 3 and group 5 with group 6.

Table 2. Comparison of animal groups by the number of mast cells depending on their degree of degranulation

Group		1st degree of degranulation. number of mast cells	2nd degree of degranulation. number of mast cells	3rd degree of degranulation. number of mast cells
Group 1: intact animals		2.64 (2.31;4.40)$^{\#,1}$	1.54 (0.88;2.53)$^{\#,1}$	0.44 (0.33;4.40)^{1}
Group 2: intact animals; a total radiation dose of 112 J/cm^2	24 hours after the experiment	1.21 (0.44;3.47)$^{\#}$	0.99 (0.44;1.38)	2.64 (1.49;3.41)$^{\circ}$
	1 week after the experiment	3.96 (1.93;6.16)	2.64 (1.60;4.35)	1.87 (1.21;3.52)
	1 month after the experiment	5.06 (4.40;6.05)$^{\#,\circ}$	4.51 (2.86;5.45)$^{\#,0}$	3.19 (1.87;5.39)
Group 3: intact animals; a total radiation dose of 450 J/cm^2	24 hours after the experiment	1.76 (1.54;2.64)	2.42 (1.43;4.07)	0.22 (0;0.94)$^{\circ}$
	1 week after the experiment	3.30 (2.15;4.68)	2.09 (1.27;3.08)	0.22 (0.17;4.24)
	1 month after the experiment	0.88 (0.55;1.43)$^{\#,\circ}$	1.98 (0.66;2.64)$^{\circ}$	1.54 (1.21;4.51)
Group 4: animals with induced hypothyroidism	24 hours after the experiment	1.54 (1.16;1.93)^{1,*}	2.42 (1.60;3.74)*	1.87 (0.99;2.75)*
	1 week after the experiment	3.63 (2.09;6.60)	2.42 (1.71;3.91)*	1.65 (1.21;3.30)*
	1 month after the experiment	3.52 (1.98;3.96)	4.84 (1.98;5.50)^{1,*}	5.94 (5.06;10.12)^{1,*}
Group 5: animals with induced hypothyroidism; a total radiation dose of 112 J/cm^2	24 hours after the experiment	4.07 (3.14;5.01)$^{*,\circ}$	4.73 (4.46;5.01)$^{*,\circ}$	8.25 (7.32;9.19)$^{*,\circ}$
	1 week after the experiment	4.29 (2.53;4.90)$^{\circ}$	3.96 (2.75;6.82)$^{\circ}$	4.07 (3.30;6.82)*
	1 month after the experiment	3.74 (3.41;4.18)$^{\circ}$	3.30 (2.75;3.63)$^{\circ}$	3.74 (2.31;6.16)*
Group 6: animals with induced hypothyroidism; a total radiation dose of 450 J/cm^2	24 hours after the experiment	1.10 (0.94;1.76)$^{\circ}$	1.98 (1.16;5.45)$^{\circ}$	2.75 (2.64;5.83)$^{\circ}$
	1 week after the experiment	1.87 (1.21;5.50)$^{\circ}$	1.21 (0.88;1.71)$^{*,\circ}$	2.86 (1.21;3.69)
	1 month after the experiment	1.65 (0.66;1.98)$^{\circ}$	1.32 (0.88;5.39)$^{*,\circ}$	2.75 (1.54;3.96)*

Notice. Data are presented as a median (lower quartile; upper quartile)

^{1} — $p < 0.05$ when comparing groups 4 and 1;
$^{\#}$ — $p < 0.05$ when comparing groups 2 and 3 with group 1;
* — $p < 0.05$ when comparing groups 5 and 6 with group 4;
° — $p < 0.05$ when comparing group 2 with group 3 and group 5 with group 6.

applied. However, there were fewer mast cells of the 1st degree of degranulation in the thyroid of the animals sacrificed 1 day after receiving a total dose of 112 J/cm^2 (Table 2). On day 30 after irradiation with a total dose of 112 J/cm^2, the total number of degranulated mast cells and mast cells of the 2nd and 3rd degrees of degranulation, as well as the value of the degranulation coefficient, were increased, but the number of the cells of the 1st degree of degranulation was low. The total number of mast cells in the thyroid of the irradiated intact animals did not change significantly on day 30 after irradiation.

Blood samples collected 24 hours after irradiation showed that laser treatment of the thyroid of the intact animals with a total dose of 112 J/cm^2 resulted in reduced TSH levels in blood serum, increased levels of unbound T_4 and increased levels of total and unbound T_3 (Table 3).

The subgroup of the hypothyroid animals who received 112 J/cm^2 irradiation and were sacrificed 24 hours after demonstrated a statistically significant increase in the total number of mast cells, the total number of degranulated mast cells of all degranulation degrees and the value of the degranulation coefficient (compared to the untreated hypothyroid rats) (Tables 1, 2) A month after laser treatment, we observed a statistically significant decrease in the total numbers of mastocytes, degranulated mast cells and mastocytes of the 3rd degree of degranulation. We also observed a statistically significant change in hormone levels: lower TSH and elevated unbound and bound T_4 and T_3 in the animals sacrificed a week and a month after irradiation.

Hypothyroid animals treated with a total dose of 450 J/cm^2 were found to have decreased levels of mast cells of the 2nd degree of degranulation a week after treatment; a month after irradiation, the number of total degranulated mast cells and mast cells of the 2nd and 3rd degrees of degranulation, the value of the degranulation coefficient and hormone levels in those animals were also low compared to the untreated hypothyroid rats (Tables 1, 2).

Comparison of different groups of animals with induced hypothyroidism who received the identical laser dose revealed that 450 J/cm^2 radiation led to the reduced number of degranulated mast cells, lower value of the degranulation coefficient and lower levels of hormones regardless of the time of sacrifice.

DISCUSSION

Thiamazole inhibits production of hormones by thyroid cells by blocking the activity of the peroxidase enzyme that mediates thyronine iodination necessary for the biosynthesis of T_3 and T_4, and thus causes hypothyroidism. Mast cells can participate in the paracrine regulation in healthy and hypothyroid subjects due to a large amount of bioactive substances contained in their granules. Degranulation of mast cells can be activated by cytokines, hormones and neuropeptides. This leads us to hypothesize that there may be an association between the functional status of stromal mast cells and the functional status of thyrocytes. This hypothesis needs to be tested.

Table 3. Levels of TSH, total (tot) and unbound (unb) thyroxine (T4) and triiodothyronine (T3) in the blood serum of experimental animals

Group		TSH (mU/l)	T4 (unb) (pmol/l)	T4 (tot) (nmol/l)	T3 (unb) (pmol/l)	T3 (tot) (nmol/l)
Group 1: intact animals		0.28 (0.21;0.75)$^{\#,1}$	18.87 (14.32;20.93)$^{\#,1}$	64.29 (39.29;138.27)$^{\#,1}$	2.09 (1.64;2.59)$^{\#,1}$	1.30 (0.96;1.39)$^{\#,1}$
Group 2: intact animals; a total radiation dose of 112 J/cm^2	24 hours after the experiment	0.19 (0.12;0.25)$^{\#,\circ}$	27.07 (22.60;27.67)$^{\#}$	72.45 (69.90;78.06)$^{\circ}$	4.63 (4.52;6.59)$^{\#,\circ}$	1.89 (1.51;2.02)$^{\#}$
	1 week after the experiment	0.21 (0.09;0.26)$^{\circ}$	19.55 (18.87;21.67)	90.82 (76.53;91.33)$^{\circ}$	4.48 (4.35;4.82)$^{\#,\circ}$	2.05 (1.81;2.47)$^{\#}$
	1 month after the experiment	0.42 (0.31;0.45)$^{\circ}$	23.87 (23.40;24.80)$^{\#,\circ}$	77.36 (75.00;78.06)	4.21 (3.59;4.77)$^{\#}$	2.10 (2.05;2.46)$^{\#}$
Group 3: intact animals; a total radiation dose of 450 J/cm^2	24 hours after the experiment	0.72 (0.49;0.83)$^{\circ}$	15.85 (12.60;24.54)	22.40 (19.09;44.41)$^{\#,\circ}$	2.53 (1.96;2.74)$^{\circ}$	1.21 (0.94;1.74)
	1 week after the experiment	1.49 (1.22;1.57)$^{\#,\circ}$	20.40 (15.59;26.66)	58.02 (46.99;62.76)$^{\circ}$	2.32 (2.21;2.48)$^{\circ}$	2.03 (1.88;2.17)$^{\#}$
	1 month after the experiment	0.10 (0.06;0.20)$^{\#,\circ}$	22.00 (18.11;23.07)$^{\circ}$	86.74 (75.00;93.37)	4.72 (4.62;4.95)$^{\#}$	2.10 (1.93;2.40)$^{\#}$
Group 4: animals with induced hypothyroidism	24 hours after the experiment	1.52 (1.27;1.75)^{1,*}	10.39 (7.61;14.87)^{1,*}	25.96 (19.59;35.04)^{1}	3.23 (3.08;3.50)^{1,*}	1.53 (1.21;1.78)^{1,*}
	1 week after the experiment	1.24 (1.17;1.39)^{1,*}	5.64 (4.62;7.78)^{1,*}	23.83 (22.32;42.35)^{1,*}	2.98 (2.39;3.14)^{1,*}	1.44 (1.32;1.95)^{1,*}
	1 month after the experiment	2.36 (2.32;2.44)^{1,*}	5.41 (5.07;8.45)^{1,*}	57.30 (41.51;77.48)*	2.74 (2.64;3.12)^{1,*}	0.99 (0.81;1.00)*
Group 5: animals with induced hypothyroidism; a total radiation dose of 112 J/cm^2	24 hours after the experiment	0.38 (0.20;0.63)*	6.92 (5.16;8.65)	30.24 (24.03;59.13)	2.96 (2.64;3.83)	1.56 (1.06;1.95)$^{\circ}$
	1 week after the experiment	0.34 (0.30;0.45)$^{*,\circ}$	10.41 (8.91;11.29)$^{*,\circ}$	51.22 (43.83;60.20)$^{\circ,*}$	3.89 (3.72;4.44)$^{*,\circ}$	2.68 (2.49;2.71)$^{*,\circ}$
	1 month after the experiment	0.19 (0.10;0.57)*	12.21 (11.80;12.97)$^{\circ,*}$	30.42 (28.74;41.43)*	3.57 (3.40;4.07)$^{*,\circ}$	1.77 (1.37;1.96)*
Group 6: animals with induced hypothyroidism; a total radiation dose of 450 J/cm^2	24 hours after the experiment	0.61 (0.36;0.71)*	6.76 (6.46;7.68)*	38.33 (32.40;48.19)	3.76 (3.51;4.20)*	2.94 (2.65;3.44)$^{*,\circ}$
	1 week after the experiment	0.51 (0.43;0.57)$^{*,\circ}$	6.52 (4.57;8.33)$^{\circ}$	13.92 (12.78;14.75)$^{\circ,*}$	2.55 (2.49;2.71)$^{\circ}$	2.14 (1.79;2.22)$^{\circ}$
	1 month after the experiment	0.42 (0.31;0.70)*	9.26 (8.65;11.56)$^{\circ}$	30.10 (29.14;34.64)*	2.96 (2.74;3.04)$^{\circ}$	1.83 (1.77;2.10)*

Notice. Data are presented as a median (lower quartile; upper quartile)

^{1} — $p < 0.05$ when comparing groups 4 and 1;
$^\#$ — $p < 0.05$ when comparing groups 2 and 3 with group 1;
* — $p < 0.05$ when comparing groups 5 and 6 with group 4;
$^\circ$ — $p < 0.05$ when comparing group 2 with group 3 and group 5 with group 6.

Based on the obtained data, we can assume that the absence of significant changes in the degranulation degree of mast cells observed in the hypothyroid animals sacrificed 24 hours after hypothyroidism induction, compared to the intact irradiated animals, is an indirect evidence of the inhibiting effect of thiamazole on the functional activity of both thyrocytes and mast cells. The increased number of mast cells of the 2nd and 3rd degrees of degranulation and the high value of the degranultation coefficient observed a month after irradiation may reflect the regenerative process in the thyroid triggered in response to the damaging effect of the thyrostatic agent and may be considered an indicator of increasing thyroid activity [10].

Lower levels of unbound T_4 and elevated levels of TSH in the hypothyroid animals (in comparison with the intact rats) regardless of the time of sacrifice indicate disease progression and suppression of the compensatory mechanisms. Elevated levels of T_3 may indicate increased T_4 deiodination in peripheral tissues aimed to restore their regulatory functions.

The observed activation of mast cells in the thyroid of the animals with induced hypothyroidism treated with a radiation dose of 112 J/cm^2 and sacrificed 24 hours after treatment, low TSH levels and increased levels of bound and unbound T_4 and T_3 a week and a month after irradiation prove that this dose stimulates mast cells and has a positive effect on the functional activity of thyrocytes in hypothyroid subjects. In contrast, at a higher radiation dose of 450 J/cm^2, mast cell degranulation was less intense and hormone levels were decreased, which leads us to conclude the activity of mast cells, as well as the activity of the entire thyroid, was inhibited.

The literature reports the stimulating effect of low-level and high-level lasers on the degranulation of mast cells in different tissues [11–13]; however, in those experiments high-level laser radiation induced thermal damage to tissues. Heating the tissue up to a temperature at which protein coagulation was observed (or to higher temperatures) activated mast cell degranulation. Tissue precooling was shown to decrease the level of mast cell degranulation [14]. The laser irradiation mode selected for our experiment did not cause any thermal damage to thyroid tissues; therefore, slower mast cell degranulation was probably due to critical laser energy doses inside the tissue. It is known that laser radiation has stimulating effects at doses below 5 J/cm^2, while higher doses have an inhibiting effect. This is however true only for superficial wounds and tissue cultures [15]. As the laser penetrates deeper tissue layers, photons are attenuated and absorbed, and the ultimate energy dose that cells receive cannot be compared to the energy dose on the skin surface [2].

Microcirculatory changes in the laser-irradiated thyroid may be associated with nitric oxide production in the irradiated area [1, 4]. Vigorous blood flow to the thyroid can be explained by the effect of laser irradiation on the mast cell degranulation which is accompanied by the release of cytokines and mediators, such as heparin, histamine, enzymes, and growth factors; in turn, sufficient blood supply is a prerequisite for the biosynthesis of iodine-containing hormones of the thyroid epithelium [8, 12, 14].

Effects of laser radiation can be explained by the absorption of photons by mitochondrial and membrane chromophores matching the wavelength of the laser, changes in the redox cell potential and processes involving secondary messengers that trigger signaling cascades inside the cell [15, 16]. In particular, mast cell degranulation after laser irradiation is traditionally associated with increasing intracellular concentrations of calcium ions [11]; therefore, decreased functional activity of mast cells may be associated with poor permeability of cell membranes to calcium resulting from the inhibiting effect of high radiation doses on calcium channels.

CONCLUSIONS

Thiamazole-induced hypothyroidism changes the levels of TSH, T_3 and T_4 in blood serum, but does not affect the functional activity of stromal mast cells of the thyroid: their response is observed only in the regeneration period when the drug is not administered any longer.

The obtained data prove that the effect of the laser on the thyroid is dose-dependent and is manifested through stimulated functional activity of mast cells and hormone-producing tissue of the thyroid. Increased functional activity of thyroid mast cells and normalized levels of hormones in blood serum in the hypothyroid animals are observed after infrared laser treatment with a total dose of 112 J/cm^2; inhibiting effects were observed at 450 J/cm^2.

Considering the above, infrared laser irradiation with a superficial dose of 112 J/cm^2 should be studied further as an antihypothyroid therapeutical technique.

References

1. Moskvin SV. Effektivnost' lazernoy terapii. Seriya «Effektivnaya lazernaya terapiya». Vol. 2. Moscow: Izd-vo "Tver"; 2015. 896 p. Russian.
2. Kravchenko TG, Zarezina AS, Golovneva ES. Otsenka glubiny proniknoveniya lazernogo izlucheniya pri terapevticheskom vozdeystvii metodom komp'yuternogo modelirovaniya. Vestnik novykh meditsinskikh tekhnologiy. 2007; 14 (2): 202–4. Russian.
3. Golovneva ES, Kravchenco TG, Kudrina MG, Gughyna AO, Popov GK. [Local effects of systemic high intensive laser radiation]. Journal of Ural Medical Academic Science. 2012; 2 (39): 126-127.
4. Hofling DB, Chavantes MC, Juliano AG, Cerri GG, Knobel M, Yoshimura EM, et al. As sessment of the effects of low-level laser therapy on the thyroid vascularization of patients with autoimmune hypothyroidism by color Doppler ultrasound. ISRN Endocrinol. 2012: 126720. DOI: 10.5402/2012/126720.
5. Hofling DB, Chavantes MC, Acencio MM, Cerry GG, Marui S, Yoshimura EM, et al. Effects of low-level laser therapy on the serum TGF-β concentrations in individuals with autoimmune thyroiditis. Photomed Laser Surg. 2014 Aug; 32 (8): 444–9. DOI: 10.1089/pho.2014.3716.
6. Morcos N, Omran M, Ghanem H, Elahdal M, Kamel N, Attia E. Phototherapeutic Effect of Low-Level Laser on Thyroid Gland of Gamma-Irradiated Rats. Photochem Photobiol. 2015 Jul–Aug; 91 (4): 942–51. DOI: 10.1111/php.12465.
7. Weber JBB, Mayer L, Cenci RA, et al. Effect of three different protocols of low-level laser therapy on thyroid hormone production after dental implant placement in an experimental rabbit model. Laser Surg. 2014; 32 (11): 612–7.
8. Aristarkhov VG. Rekomendatsii po primeneniyu infrakrasnogo lazernogo izlucheniya u bol'nykh s patologiey shchitovidnoy zhelezy. In: Materialy nauchno-prakticheskoy konferentsii GBOU VPO RyazGMU Minzdrava Rossii; 2014; Ryazan, Russia. Ryazan: RIO RyazGMU; 2014. Russian.
9. Yushkov BG, Chereshnev VA, Klimin VG, Artashyan OS. Tuchnye kletki. Fiziologiya i patofiziologiya. Moscow: Izd-vo "Meditsina"; 2011. 240 p.
10. Artashyan OS, Khramtsova YuS, Yushkov BG. [Participation of

mast cells in process of reparative regeneration of the thyroid]. Journal of Ural Medical Academic Science. 2013; 44 (2): 36–8. Russian.
11. Kozel AI, Soloveva LI, Popov GK. K mekhanizmu deystviya nizkointensivnogo lazernogo izlucheniya na kletku. Byulleten' eksperimental'noy biologii i meditsiny. 1999; 128 (10): 397–9. Russian.
12. Golovneva ES, Kozel AI, Popov GK. [Pathophysiological mechanism of tissue revascularization by using an impact of a high-intensity laser irradiation]. Annals of the Russian academy of medical sciences. 2003; (6): 23–6. Russian.
13. Isman CA, Yegen BC, Alican I. Methimazole-induced hypothyroidism in rats ameliorates oxidative injury in experimental colitis. J Endocrinol. 2003; 177 (3): 471–6.
14. Pinheiro AL, Browne RM, Frame JW, Matthews JB. Mast cells in laser and surgical wounds. Braz Dent J. 1995; 6 (1): 11–5.
15. Chaves ME, Araujo AR, Piancastelli AC, Pinotty M. Effects of low-power light therapy on wound healing: LASER x LED. An Bras Dermatol. 2014 Jul–Aug; 89 (4): 616–23.
16. Zalesskiy VN. K 50-letiyu lazernoy meditsiny: molekulyarnye mekhanizmy lazernoy biostimulyatsii. Ukrainskiy meditsinskiy zhurnal. 2010; 5 (79): 52–8. Russian.

Литература

1. Москвин С. В. Эффективность лазерной терапии. Серия «Эффективная лазерная терапия». Т. 2. М.: Изд-во «Тверь»; 2015. 896 с.
2. Кравченко Т. Г., Зарезина А. С., Головнева Е. С. Оценка глубины проникновения лазерного излучения при терапевтическом воздействии методом компьютерного моделирования. Вестник новых медицинских технологий. 2007; 14 (2): 202–4.
3. Головнева Е. С., Кравченко Т. Г., Кудрина М. Г., Гужина А. О., Попов Г. К. Локальные эффекты системного лазерного облучения повышенной мощности. Вестник уральской академической науки. 2012; 2 (39): 126-127.
4. Hofling DB, Chavantes MC, Juliano AG, Cerri GG, Knobel M, Yoshimura EM, et al. As sessment of the effects of low-level laser therapy on the thyroid vascularization of patients with autoimmune hypothyroidism by color Doppler ultrasound. ISRN Endocrinol. 2012: 126720. DOI: 10.5402/2012/126720.
5. Hofling DB, Chavantes MC, Acencio MM, Cerry GG, Marui S, Yoshimura EM, et al. Effects of low-level laser therapy on the serum TGF-β concentrations in individuals with autoimmune thyroiditis. Photomed Laser Surg. 2014 Aug; 32 (8): 444–9. DOI: 10.1089/pho.2014.3716.
6. Morcos N, Omran M, Ghanem H, Elahdal M, Kamel N, Attia E. Phototherapeutic Effect of Low-Level Laser on Thyroid Gland of Gamma-Irradiated Rats. Photochem Photobiol. 2015 Jul–Aug; 91 (4): 942–51. DOI: 10.1111/php.12465.
7. Weber JBB, Mayer L, Cenci RA, et al. Effect of three different protocols of low-level laser therapy on thyroid hormone production after dental implant placement in an experimental rabbit model. Laser Surg. 2014; 32 (11): 612–7.
8. Аристархов В. Г. Рекомендации по применению инфракрасного лазерного излучения у больных с патологией щитовидной железы. В сб.: Материалы научно-практической конференции ГБОУ ВПО РязГМУ Минздрава России; 2014 г.; Рязань. Рязань: РИО РязГМУ; 2014.
9. Юшков Б. Г., Черешнев В. А., Климин В. Г., Арташян О. С. Тучные клетки. Физиология и патофизиология. М.: Изд-во «Медицина»; 2011. 240 с.
10. Арташян О. С., Храмцова Ю. С., Юшков Б. Г. Участие тучных клеток в процессе репаративной регенерации щитовидной железы. Вестник уральской медицинской академической науки. 2013; 44 (2): 36–8.
11. Козель А. И., Соловьева Л. И., Попов Г. К. К механизму действия низкоинтенсивного лазерного излучения на клетку. Бюллетень экспериментальной биологии и медицины. 1999; 128 (10): 397–9.
12. Головнева Е. С., Козель А. И., Попов Г. К. Патофизиологический механизм реваскуляризации тканей с помощью воздействия высокоинтенсивного лазерного излучения. Вестник Российской академии медицинских наук. 2003; (6): 23–6.
13. Isman CA, Yegen BC, Alican I. Methimazole-induced hypothyroidism in rats ameliorates oxidative injury in experimental colitis. J Endocrinol. 2003; 177 (3): 471–6.
14. Pinheiro AL, Browne RM, Frame JW, Matthews JB. Mast cells in laser and surgical wounds. Braz Dent J. 1995; 6 (1): 11–5.
15. Chaves ME, Araujo AR, Piancastelli AC, Pinotty M. Effects of low-power light therapy on wound healing: LASER x LED. An Bras Dermatol. 2014 Jul–Aug; 89 (4): 616–23.
16. Залесский В. Н. К 50-летию лазерной медицины: молекулярные механизмы лазерной биостимуляции. Украинский медицинский журнал. 2010; 5 (79): 52–8.