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Morphofunctional changes in the canine thyroid gland under low-intensity radiation exposure

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Abstract

The results of studies of morphological changes in the thyroid gland and the functional state of the organism of clinically healthy dogs living in the III zone of radioactive contamination (III RCZ) after a man-made accident at a nuclear power plant are presented. The aim of the work is to study the effect of prolonged radioactive radiation of caesium–137 radionuclides on changes in the morphofunctional state of the thyroid gland. Clinical, hematological, biochemical and pathomorphological studies of animals were carried out, the degree of radioactive contamination of water, feed, the background of the environment, the specific activity of gamma radiation of thyroid tissues were studied. The radiological indicators of the III zone of radioactive contamination are 3 times higher in terms of the power of the exposure dose of radiation and 6.8 times higher in terms of the specific activity of the diet and thyroid tissues. Abnormalities of adequate metabolism and functional activity of different systems were found in clinically healthy dogs. Signs of hypothyroidism were clinically identified in 28% of dogs. Laboratory studies have established hypochromic anemia, leucopenia, hypoalbuminemia, bilirubinemia, aspartate aminotransferase hyperfermentation, creatininemia, uremia, and cholesterolemia. In the hormonal profile, the level of triiodothyronine, thyroxine and thyroid-stimulating hormone was significantly reduced, which indicates the development of secondary hypothyroidism under the influence of caesium-137. Pathomorphological studies have determined hyperplasia of the organ, degenerative processes in functional structures – follicles, desquamation and degradation of thyrocytes, hemorrhages in the interfollicular tissue, which is the morphological equivalent of hypothyroidism.

Key Words: caesium-137, hypothyroidism, ionizing radiation, thyrocytes, thyroid hormones

Introduction

In the conditions of the modern world, the use of technological systems is observed, which over time become more and more complex. The number of various man-made accidents and catastrophes is significantly increasing in the world[30)]. Accidents at nuclear power plants, as well as other nuclear cycle facilities, have both near-term and long-term consequences[25)]. The consequences of the Chernobyl accident are characterized by significant environmental pollution with caesium-137(Cs-137) radionuclides[18)]. These isotopes have a wide pathophysiological effect on the body of animals, including the thyroid gland, an endocrine organ that plays an important role in the regulation of metabolism and the functioning of the body as an integral system. The hormones of this gland – calcitonin, triiodothyronine (T3) and thyroxine (T4) – regulate the work of various

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organs and systems. The gland is controlled by the pituitary gland by the synthesis of thyroid-stimulating hormone (TSH)[5,16]. The accumulation of iodine-131 in thyroid gland is widely known. However, in the long-term periods after accident, the influence of this isotope is not relevant.

The aim of the work is to study the effect of low–intensity prolonged radioactive radiation of nuclear cycle products (Cs-137) on the morphological structures and functions of the thyroid gland.

Materials and Methods

The studies were conducted in the period 2018–2022.

Ethics Committee approval was received for this study from the Ethics Committee of Polissia National University, Zhytomyr, Ukraine (Approval number: 2019/07, 2020/10).

Sampling

Domestic mongrel dogs were used, age 1 year, the average body weight is 20-30 kg. The choice of such an age category is due to the maximum functional activity of the thyroid gland in the formation of metabolic regulation.

Metadatas on the clinical condition of dogs were studied in groups of dogs, including 100 animals in each study area.

For laboratory studies, the study and control groups were formed according to the principle of pairs-analogues, 20 animals each.

The study group included clinically healthy animals living in the territory of the III zone of radioactive contamination (III RCZ). The criteria of clinically healthy animals were the absence of pronounced clinical signs of impaired functional activity of organs and systems, the absence of identified pathogens of infections. The control group consists of dogs of a similar age which live in a relatively clean area of radioactive contamination (CZ).

During the pathomorphological studies, 2 groups of 6 individuals were formed in each. All animals were clinically healthy and died from cases of massive injuries incompatible with life during the research period. The study group is dogs of III RCZ. Control group is dogs of CZ.

Radiological studies

The degree of radioactive contamination of water, feed, and the background of the environment was determined by a dosimeter-radiometer Ecotest MKS-05 "Terra" (Ecotest, Ukraine).

The specific activity of gamma radiation of thyroid tissue was studied on a spectrophotometer SEG-001 AKP-C-63 (AtomComplexPrilad, Ukraine). The equivalent dose was calculated using the dose coefficient for Cs-137.

Clinical studies, hematological and biochemical researching

Clinical studies made by General methods of clinical research.

Blood samples for laboratory studies were taken from the right *vena subcutanea antebrachii*. For hematological studies, Vacumed (Micromed®, Italy) tubes with the anticoagulant EDTA-K3, the concentration of which is 1.8 mg/ml, were used. For biochemical studies and for enzyme immunoassay, whole blood samples taken in Vacuette tubes (Greiner Bio-one®, Austria)) with a clot activator and a delimiting gel were used. Blood serum was obtained, which served as a material for study.

The cytological composition of the blood and the hemoglobin content were determined on a hematological analyzer MicroCC-20Plus (HTI, USA).

Among the biochemical parameters, the level of total bilirubin, creatinine, urea, the activity of aspartate aminotransferase AsAT (EC 2.6.1.1.), gamma-glutamyltranspeptidase GGT (EC 2.3.2.2.), cholesterol in blood plasma were studied. An automatic biochemical analyzer BioChem SA (HTI, USA) was used.

Hormonal researching

To study the level of hormones TSH, T3, T4, the method of immunosorbent analysis using fixed enzymes on a multifunctional microplate

Table 1. Exposure dose rate of radioactive radiation of dog habitat areas

Units of measurement	Exposure dose	
	III RCZ	CZ
nCl/kg/hr	11.8 ± 1.4 ***	3.2 ± 0.15

Note: *** $P < 0.001$ relative to the control group

Table 2. Specific activity of the diet, thyroid gland (Bq/kg) and the equivalent dose of ionizing radiation energy absorption (μSv) according to Cs-137

Measurement object	III RCZ	CZ
Diet, Bq/kg	382 ± 8.6***	56.3 ± 5.2
Thyroid gland, Bq/kg	213 ± 11.3 ***	31.4 ± 3.0
Equivalent dose: μSv/hr	0.41 ± 0.01 ***	0.08 ± 0.016
μSv/day	9.8 ± 0,3 ***	1.9 ± 0.35
μSv/year	3500 ± 120 ***	700 ± 120

Note: *** $P < 0.001$ relative to the control group

photometer Immunochem-2 (High Technology Inc., USA) was used.

Organometrical and pathomorphological researching

The thyroid gland was extracted from the body of the deceased animal. An autopsy of the skin in the area of the first rings of the trachea was performed, the thyroid gland was dissected. The linear dimensions, absolute and relative mass of the organ were measured.

Pieces of organs were poured into paraffin and histological sections were made on a sled microtome HM 430 (Microm®, Germany).

Hematoxylin-eosin staining was performed according to Van Gieson. Microphotography was performed using a Micros MC-50 Lotus digital microscope with a camera (Micros, Austria).

Statistical analysis

Statistical processing of the obtained results was carried out by ANOVA analysis of variance using the StatSoft Statistica Analyst 14 IT application. Reliability was established using Fisher's F-test.

Table 3. Laboratory blood parameters of dogs

Parameter	Study group	Control group
Hemoglobin, g / l	95.6 ± 3.8 ***	140.2 ± 3.8
Red blood cells, T / l	5.35 ± 0.2 ***	7.7 ± 0.19
White blood cells, G / l	6.2 ± 0.15 ***	9.2 ± 0.35
Albumins, g/l	23.8 ± 1.7 ***	34.1 ± 2.5
Total bilirubin, mmol/l	6.3 ± 1.2 ***	3.8 ± 0.06
Creatinine, μmol/l	127 ± 8.4 ***	92 ± 4.2
Urea, mmol/l	7.3 ± 0.7 ***	4.2 ± 0.18
AsAT, U/l	64.3 ± 4.2 ***	19.3 ± 2.1
GGT, U/l	2.8 ± 0.01	3.2 ± 0.6
Cholesterol, mmol/l	10.2 ± 1.6 ***	3.2 ± 0.32

Note: *** $P < 0.001$ relative to the control group

Results

Taking into account a sufficiently long time after the accident at the nuclear power plant, the radiation background in the study area is relatively the same and has slight fluctuations in different seasonal periods, which do not go beyond the measurement error. The radiological assessment of the study areas is represented by indicators of the exposure dose rate (Table 1).

III RCZ is characterized by the exposure dose rate of radioactive radiation 3 times higher than the CZ.

Radionuclides enter the animal body with feed and water[18,21,24,27]. During the year, the diet of dogs is of the same type – it is waste after the slaughter of animals in slaughterhouses and meat processing plants, sometimes cereal porridge. The indicator of the specific activity of thyroid tissues was recalculated into the equivalent dose of absorption of ionizing radiation energy according to Cs-137 (Table 2).

The diet of dogs and thyroid tissue in III RCZ have a specific activity of Cs-137 6.8 times higher than that of CZ. The estimated equivalent dose of absorbed ionizing radiation energy by thyroid tissue is 5 times higher, respectively.

According to the results of clinical studies, changes were found (% of the total number of studied) (Fig. 1).

Laboratory studies add depth to the

Table 4. The level of thyroid hormones in the blood of dogs from different zones of radioactive contamination

Parameter	Study group	Control group
Thyroid-stimulating hormone, mU/l	1.6 ± 0.04***	4.8 ±0.08
Free thyroxine, nmol/l	10.6 ± 1.2***	25.6 ± 3.2
General triiodothyronine, nmol/l	0.2 ± 0.03***	1.8 ± 0.05

Note: *** $P < 0.001$ relative to the control group

Table 5. Morphometric parameters of the thyroid gland of 1-year-old dogs in different zones of radioactive contamination

Parameter	Animal group	
	study	control
Gland relative mass, %	0.007 ± 0.001***	0.011 ± 0.001
Average follicle diameter, μm	143.3 ± 4.7***	110.3 ± 4.6
including:large follicle diameter, μm	233.6 ± 6.2**	207.2 ± 5.3
middle follicle diameter, μm	158.7 ± 4.1**	139.8 ± 5.1
small follicle diameter, μm	90.3 ± 2.8**	71.6 ± 2.8
Specific area of follicles per 5 mm^2, mm^2 (%)	4.2 ± 0.02*** (77 ± 1.2***)	4.5 ± 0.03 (88 ± 0.8)
Specific area of the interfollicular part per 5 mm^2, mm^2 (%)	1.21 ±0.045*** (22.2 ± 0.8***)	0.55 ± 0.02 (13.6 ± 0.5)

Note: **$P < 0.01$; *** $P < 0.001$ relative to the control group

characteristics of the general condition of dogs (Table 3).

The dogs of the study group had significant hypochromic anemia, leukopenia, hypoalbuminemia, bilirubinemia, creatininemia, uremia, AsAT hyperfermentation, and cholesterolemia.

The functional activity of the thyroid gland can be assessed by the level of thyroid hormones (Table 4).

In animals of the study group, the levels of TSH, T3 and T4 are significantly reduced and the indicators go beyond physiological limits.

Pathomorphological studies have established that in animals of the control group, the consistency of the thyroid gland is dense, the color is dark red. The organ particles have an oval shape, flattened on both sides. The surface of the gland is smooth. The follicles in the middle are lined with cubic thyrocytes. Inside the follicle is a colloid with resorption vacuoles. The dominant shape of the follicles is oblong (Fig. 2).

In dogs of the study group, thyrocytes are

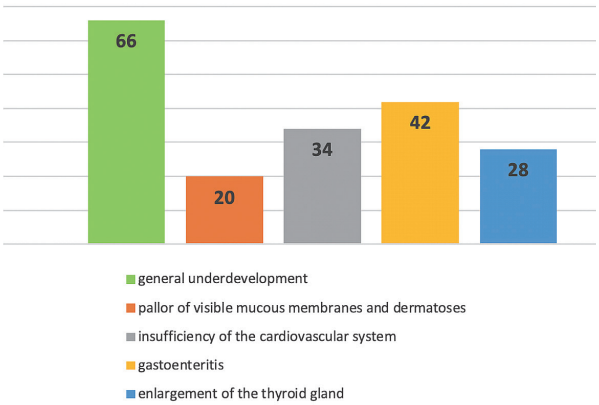

Fig. 1. Frequency of occurrence of the main clinical signs in animals of the III RCZ (%).

flat and desquamated. Most follicles do not contain a colloid. Some follicles contain a small amount of condensed colloid. Vascular penetration and numerous hemorrhages were noted in the interfollicular tissue (Fig. 3).

Morphometric parameters of the canine thyroid gland of the study group in comparison with those of the control group were significantly changed (Table 5).

The dimensions of the structural components of the gland are increased, but the specific area of the functional unit of the gland – follicles is significantly reduced.

Discussion

The prolonged effect of low doses of ionizing radiation of nuclear cycle products on the animal body leads to destructive morphological changes in the different organs including thyroid gland. This gland plays an important role in the regulation of metabolism[2,4,9,12,17].

Dogs in certain ecological zones feed mainly on feeds of local origin. In the III RCZ, the feed constantly accumulates radionuclides, mainly Cs-137[27].

The exposure dose rate for gamma radiation in III RCZ is 3 times higher compared to the clean zone (Table 1). The specific activity of the diet is 6.8 times higher (Table 2).

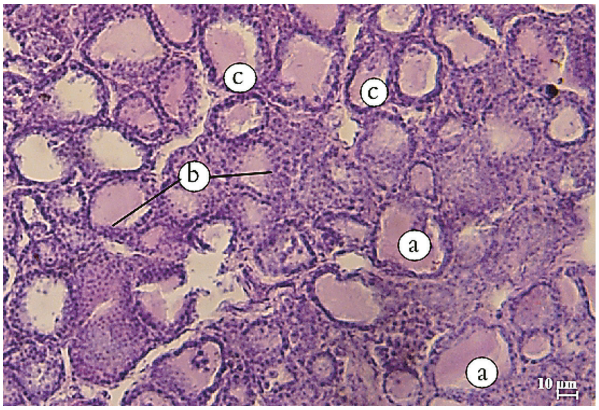

Fig. 2. Morphological structure of the thyroid gland in a 1-year-old dog from the clean zone: a – colloid, b – thyrocytes, c – oblong follicle. Hematoxylin-eosin, × 200.

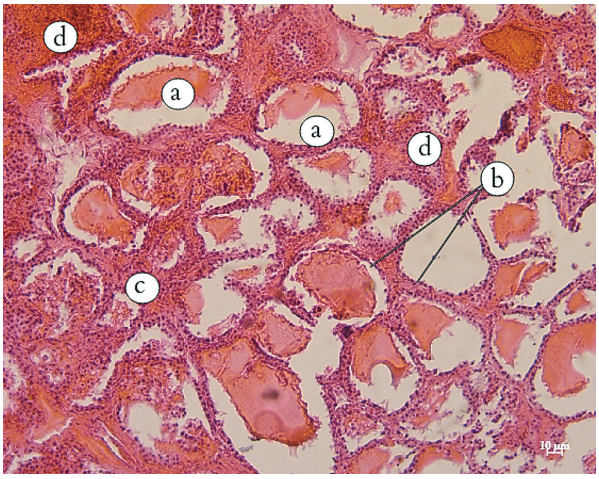

Fig. 3. Morphological structure of the thyroid gland of a 1-year-old dog from the III RCZ: a – follicle with condensed colloid; b – follicle thyrocytes, flat and desquamated; c – interfollicular tissue; d – hemorrhages in the interfollicular tissue. Hematoxylin-eosin. × 200.

It is known[24,27], that Cs-137 has a pronounced cumulation effect. The specific activity of thyroid tissue is also 6.8 times higher in dogs of III RCZ (Table 2).

In clinically healthy dogs of the study group, under conditions of normal visible functioning of the body, developmental disorders, circulatory insufficiency (anemia of the mucous membranes), dermatoses, chronic cardiovascular insufficiency, disorders of the gastrointestinal tract were found (Fig. 1). Thus, animals develop with abnormalities in adequate metabolism[3,11,15,29].

The determining regulator of metabolism in animals is the thyroid gland. It, in turn, is controlled by the influence of pituitary TSH[16].

In 28% of dogs, clinical signs of hypothyroidism were established, which manifested themselves in an increase in the thyroid gland, which can be palpated, as well as enophthalmos. Such symptoms may be characteristic of endemic goiter, which is a regional pathology in the conditions of the biogeochemical zone of iodine deficiency [6-8,22,23]. In the control group of dogs, the signs described above have not been established.

During laboratory tests (Table 3), hypochromic anemia was found, which is caused by hypothyroidism. With it, due to disorders of the gastrointestinal tract, the absorption of iron, vitamin B12 and folic acid, which are so important for normal erythropoiesis, is disrupted[20,26]. The main regulator of the adequate functioning of the intestine and the absorption of these elements is the hormone thyroxine. Given the chronic nature of anemia and, as a consequence, chronic hypoxia, conditions for the development of various diseases are created in the dog's body of III RCZ[8,23,26].

Pronounced leukopenia indicates the depressing effect of radionuclides on leukopoiesis. This condition leads to a decrease in the protective functions of the body and, as a result, to an increase in the incidence of both internal and infectious diseases.

Biochemical blood parameters indicate damage to hepatocytes (hypoalbuminemia, bilirubinemia, AsAT hyperfermentation). The work of these structures is regulated by T3 and T4. There is also a feedback relationship between hepatocytes and these hormones. Thus, the insufficiency of hepatocytes increases the insufficiency of the thyroid gland[7,8,11,19].

Creatininemia and uremia indicate the development of renal failure[10,28]. Filtration and resorption activity of the kidneys is regulated by thyroid hormones.

Pronounced cholesterolemia indicates a violation of lipid metabolism, which also depends on the hormonal regulation of the thyroid gland. Due to insufficient production of thyroid hormones, the lipogenic activity of the

liver decreases, as a result of which cholesterol accumulates in the blood[14].

The hormonal profile in dogs (Table 4) of RCZ III is characterized by a significant decrease in all indicators. With the development of endemic primary hypothyroidism, a decrease in T3 and T4 levels occurs against the background of an increase in TSH levels. In our studies, hypothyroidism has a secondary origin and is caused, among other things, by the depressing effect of ionizing radiation from accumulated in the thyroid gland radionuclides on the synthesis of TSH. Thus, secondary hypothyroidism develops in animals under conditions of prolonged intake of radionuclides into the body[7,8,13].

As a confirmation, a pathomorphological examination of the dogs thyroid gland of III RCZ was performed (Table 5). Signs of organ hyperplasia have been established – the organ is enlarged in size due to an increase in the ratio of interfollicular tissue / follicles, degenerative processes in functional structures – follicles, namely desquamation and degradation of thyrocytes, colloid condensation (Fig. 3), which determines the impossibility of adequate synthesis of hormones T3 and T4, which are so necessary for normal metabolism[1,4,15].

Thus, in dogs of III RCZ, morphofunctional changes in the thyroid gland under the influence of Cs-137 radionuclides are biochemical and pathomorphological equivalents of secondary hypothyroidism. The influence of ionizing radiation leads to significant changes in the morphological structure of the organ, which in turn leads to further violations of the vital functions of the body.

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