Comparative Efficiency of Low-Esterified Pectin and Antistrumin during Experimental Hypofunction of the Thyroid Gland

Yu. S. Khotimchenko and I. S. Sergushchenko*

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We studied the effects of low-esterified pectin and antistrumin in rats with hypofunction of the thyroid gland modeled by enteral administration of lead acetate. Pectin more rapidly recovered thyroid function than iodine.

Key Words: *thyroid gland; lead; pectin; antistrumin*

The development of thyroid hypofunction during relative iodine deficiency is related to the increase in the content of toxic compounds, including heavy metals, in the environment and human organism [6].

Chemical pollutants impair the synthesis of intrathyroid hormones, suppress thyroxine conversion into triiodothyronine in peripheral tissues, and affect cell receptors for thyroid hormones [8,11]. The amount of thyroid hormones decreases, while the concentration of thyrotropic hormone (TTH) increases in workers with high level of lead in the blood [12,13]. Experiments on animals showed that pathological structural changes in the thyroid gland modeled by enteral administration of toxicants are accompanied by intensive secretion of TTH [14].

Traditional methods for the therapy and prevention of iodine deficiency with iodine in microdoses are low efficient in people living under unfavorable environmental conditions and exposed to the influence of chemical toxicants on the thyroid gland. It is necessary to develop new approaches to differential therapy of endemic goiter that depend on the nature of xenobiotics affecting the thyroid gland [4].

Laboratory of Pharmacology, Institute of Sea Biology, Far-Eastern Division of the Russian Academy of Sciences; *Department of Pharmacology, Vladivostok State Medical University. ***Address for correspondence:*** inmarbio@mail.primorye.ru. Khotimchenko Yu. S.

We assumed that efferent therapeutic agents capable of binding and eliminating toxic compounds would produce the positive effect during ecological relative iodine deficiency. Plant polysaccharides belonging to the class of pectins with enterosorption activity possess these properties [5]. Metal-binding activity of pectins depends on their structure and physicochemical properties and markedly increases with decreasing the degree of esterification [2,10]. Here we compared the efficiency of low-esterified pectin and iodine-containing preparation antistrumin during experimental hypofunction of the thyroid gland modeled by enteral administration of lead acetate.

MATERIALS AND METHODS

Preparations of low-esterified pectin were obtained from highly-esterified pectin not containing acetyl and amid groups (Copenhagen Pectin A/S, Lille Skensved) by alkaline deesterification. Commercial highly-esterified pectin (100 g) was treated with 1600 ml 50% ethanol containing 20 g NaOH at 20°C for 30 min, neutralized with 10% HCl, filtered, and dried at 60°C (final humidity <4%). Galacturonic acid content in the pectin was measured by the colorimetric hydroxydiphenyl method [7]. The degree of pectin esterification was estimated titrimetrically [1]. Characteristic viscosity was determined in the mixture of 0.05 M NaCl and 0.005 M sodium oxalate at 25°C and pH 6.0 using an Ubbelode viscometer. The sample of pectin had the following physicochemical characteristics: galacturonic acid content 67.1%, esterification 2.5%, and characteristic viscosity 209 ml/g galacturonic acid.

Experiments were performed on male outbred albino rats weighing 130-160 g. The animals were kept in wire cages at 20-22°C and relative humidity 60-65%. They fed a diet containing (per 100 g) 21.0 g casein, 5.3 g cellulose, 7.0 g sunflower oil, 1.0 g cholesterol, 15.0 g sucrose, 45.9 g starch, 0.3 g methionine, 0.3 g mineral components, and 1.0 g vitamins. The rats were maintained according to instructions for care of experimental animals. The animals were divided into 5 groups. Group 1 and 2 rats were divided into 2 subgroups. Group 1 animals fed a standard diet (control). Other animals received lead acetate in a daily dose of 100 mg/kg through a metal gastric tube 30 min before feeding for 21 days. Some animals were used for morphological and biochemical assays (subgroups control-1 and lead-1). The rats of groups 1 (subgroup control-2) and 2 (subgroup lead-2) fed only the standard diet for the next 21 days. Group 3 animals received antistrumin in a dose of 10 μg/kg immediately before feeding (through a gastric probe). Low-esterified pectin (0.1 g/kg, 1% solution) was administered intragastrically to group 4 rats 60 min before feeding. Group 5 animals received pectin and antistrumin in the same doses; pectin was given 60 min before antistrumin administration. The animals were decapitated by the end of the experiment [3]. The thyroid glands were removed and fixed for histological examination and quantitative measurement of lead. Slices (5 μ) were stained with hematoxylin and eosin. Images of micropreparations were visualized on a computer using a Vickers-85 microdensitometer equipped with a video system. Digital image processing was performed by means of Adobe Photoshop 5.0 and Microsoft Excel 97 softwares. The height of the follicular layer, relative area of follicles, and pathological structural changes was determined in several fields of view. Lead content in the thyroid gland was measured by the method of atomic absorption spectrophotometry [9]. The concentrations of thyroxine and triiodothyronine in the plasma were estimated by enzyme immunoassay with T_3-IFA-Best-strip and T_4-IFA-Best-strip test systems (Vektor Best).

The results were analyzed by Student's *t* test.

RESULTS

Administration of lead acetate for 21 days was followed by an increase in lead content in the thyroid gland and decrease in the weight of the thyroid gland (by 5.3 times and 38.3%, respectively). These changes were accompanied by a decrease in plasma concentrations of thyroxine and triiodothyronine. Histological examination revealed signs of atrophy in the thyroid gland. Most follicles had irregular shape. Their wall formed a considerable number of folds and invaginations due to hyperplasia of the follicular epithelium. The lumen of follicles was filled with a weakly colored colloid vacuolized in peripheral and central regions. The wall of follicles was lined with the cubical or prismatic epithelium. Several epitheliocytes formed "pillows" (multilayered overgrowths of thyrocytes into the lumen of follicles). Agglomerates of thyrocytes completely obliterated the lumen of follicles. Central regions of the thyroid gland contained overgrowths of the connective tissue, which occupied the interfollicular space and often grew into follicles. Necrotic

TABLE 1. Effects of Low-Esterified Pectin and Antistrumin on the State of Thyroid Gland and Blood Level of Thyroid Hormones in Rats with Lead Poisoning ($M \pm SEM$)

Group	Weight of thyroid gland, mg/100 g	Lead, μg/g dry gland weight	Height of follicular epithelium, μ	Area of epithelium, % of the area of follicles (slice)	Area of follicles, % of the area of gland (slice)	Triiodo-thyronine, ng/ml	Thyroxin, ng/ml
Control-1, *n*=6	12.8±1.1	7.3±2.1	6.3±0.2	32.6±2.5	60.1±7.4	2.78±0.17	34.76±3.93
Lead-1, *n*=5	7.9±0.7*	38.3±2.9*	10.2±0.3*	46.8±3.6*	37.6±6.4*	1.58±0.21*	23.14±2.79*
Control-2, *n*=6	14.2±0.6	6.3±1.9	6.2±0.2	34.5±2.8	58.8±7.8	2.95±0.27	31.20±4.81
Lead-2, *n*=6	7.6±0.4$^+$	35.0±3.2$^+$	9.0±0.3$^+$	44.7±3.0$^+$	34.6±6.1$^+$	2.02±0.16$^+$	22.03±1.19$^+$
Antistrumin, *n*=8	8.7±0.3	36.4±3.8	7.6±0.3^o	37.5±3.1	38.5±8.2	2.29±0.13	23.72±1.07
Pectin, *n*=8	15.7±1.8^o	17.5±2.2^o	5.7±0.2^o	31.8±3.0^o	64.3±6.7^o	2.67±0.24^o	22.09±1.31
Antistrumin and pectin, *n*=6	16.5±0.7^o	21.8±2.8^o	6.1±0.3^o	32.8±3.2^o	78.9±7.4^o	2.48±0.22	26.14±1.56

Note. $p<0.05$: *compared to control-1; $^+$compared to control-2; ^{o}compared to lead-2.

areas appeared as zones of enucleated amorphous material. The relative area of follicular epithelium increased and surpassed that of follicles. The number of follicles decreased in slices of the thyroid gland (Table 1). These changes reflected the increase in functional activity (including regenerative activity) of follicles against the background of pronounced sclerotic and necrotic changes in the greater area of the thyroid gland.

Changes in the thyroid gland persisted for 21 days after the last treatment with lead acetate. The relative weight and morphometric indexes of the thyroid gland and concentration of thyroid hormones in the plasma remained unchanged. Antistrumin significantly decreased the height of the follicular layer in group 3 rats. Weakly stained colloid was vacuolized mainly in the peripheral zones of the follicle. Other indexes did not differ in rats of groups 3 and 2 (lead-2). In group 4 animals receiving pectin the weight of the thyroid gland returned to normal, and lead content decreased by 2 times. Examination of micropreparations revealed round follicles with normal size that included brightly colored colloid. The observed changes reflected an increase in the concentration of thyroglobulin. The height of the follicular epithelium presented by prismatic cells decreased to normal. Signs of connective tissue overgrowth were less pronounced. The relative size of follicles in these rats reached the control level and differed from that in animals of subgroup lead-2. Triiodothyronine level surpassed that in rats of subgroup lead-2. However, plasma thyroxine concentration remained low. Probably, a longer recovery period is required for reducing thyroxine content to normal. Microscopic indexes of the thyroid gland in group 5 rats receiving pectin and antistrumin were similar to those in control animals. In central regions of the thyroid gland small follicles had narrow cavities and were lined with multilayered cubical or prismatic epithelium with round nuclei. Therefore, the recovery of structural characteristics in the thyroid gland was related to activation of regenerative processes. The test parameters in animals of this group returned to normal (except for blood hormone level). Lead content in the thyroid gland of rats receiving pectin alone or in combination with antistrumin decreased, but surpassed that in animals not treated after lead load (by 2.5 and 3.5 times, respectively).

Our results suggest that substances binding heavy metals more rapidly recover thyroid hypofunction after lead poisoning compared to iodine preparations. Moreover, structural characteristics of the thyroid gland rapidly return to normal after combination treatment with sorbents and iodine preparations. The optimal doses and duration of treatment with preparations will be evaluated in further studies.

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