Original Article

Effects of 60-day Sodium Benzoate Exposure on the Ultramicroscopic Structure of Rat's Thyroid Gland Follicular Cells

Abstract

Background: The sodium benzoate is widely used as a preservative in the food and pharmaceutical industries. There is information about the ability of this food additive to cause hepato- and nephrotoxicity, gonadotoxicity by initiating oxidative stress in cells. However, information on the effect of long-term exposure to sodium benzoate on the ultrastructure of endocrine cells, particularly of those thyroid follicular cells is completely absent. **Aim:** The aim of the study was to investigate electron microscopic changes in thyroid gland follicular cells in mature rats after 60 days of sodium benzoate administration. **Materials and Methods:** The first group consisted of rats injected daily with 1 ml sodium benzoate solution at a dosage of 1000 mg/kg of body weight through a feeding tube for 60 calendar days. The second group consisted of control animals injected with 0.9% saline solution under the same conditions. Thyroid gland pieces were processed according to the standard protocol for electron microscopy. **Results:** Microscopic examination of the thyroid gland of experimental animals showed that the follicular cells had a cubic or flattened shape. The apical surface of these cells contained a small number of low microvilli. The rough endoplasmic reticulum (rER) of most thyrocytes was enlarged and its cisterns contained homogeneous material, but some had abnormal electron dense deposits. In most cells, the nuclei were often irregular in shape with irregular contours, compared to the control. Heterochromatin occupied almost the entire periphery of the karyoplasm. The mitochondrial matrix was electron dense. Lysosomes were evenly distributed in the cytoplasm. A small number of small pinocytic vesicles with colloids were located in the apical part of most thyrocytes. A wide electron light pericapillary space was observed between the basal part of the latter and fenestrated capillaries. **Conclusion:** The 60 day administration of sodium benzoate to rats causes adverse ultrastructural changes in thyroid follicular cells. The rER undergoes the greatest morphological changes.

Keywords: *Follicular cells, rats, sodium benzoate, thyroid gland, ultrastructure*

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Introduction

At present, sodium benzoate is widely used as a preservative for food and beverages, in the pharmaceutical industry. It can be found as part of canned food, dairy and meat products, seasonings, and semi-finished products, as well as in the outer shell of drugs and personal hygiene products.[1] However, concerns remain about its complete safety for human health. The experimental and clinical studies show ambiguous results in this regard.

Thus, there is experimental evidence that the introduction of sodium benzoate causes chromosomal aberrations in cultured human lymphocytes;[2] induces the development of oxidative stress, reduces the activity of enzymes of the antioxidant system, increases the level of liver biochemical markers (alanine aminotransferase and aspartate aminotransferase) and pro-inflammatory cytokines (interleukin 6, tumor necrosis factor-alpha) in cells.[3]

According to other sources, sodium benzoate has no mutagenic and teratogenic effects[4] and is also used in clinical practice in the therapy of resistant forms of schizophrenia, dementia, chronic liver diseases, and Parkinson's disease.[5-8]

The endocrine system, together with the nervous system, ensures the regulation and coordination of body functions and is one of the first to be exposed to adverse factors of both the external and internal environment.[9]

Physical and emotional health requires the normal functioning of the thyroid gland, which regulates the body's executive systems, metabolic rate, behavior, and cognitive function. The earliest

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morphological changes under the influence of unfavorable factors are found at the subcellular and cellular levels of the organization of matter. The aim of the study was to investigate electron microscopic changes in thyroid gland follicular cells in mature rats after 60 days of administration of sodium benzoate.

Subjects and Methods

For the experiment, 12 white male mature rats weighing 200–250 g were selected. The animals were divided into two groups of six individuals each. The first group consisted of rats injected daily with 1 ml sodium benzoate solution at a dosage of 1000 mg/kg of body weight through a feeding tube for 60 calendar days. The second group consisted of control animals injected with 0.9% saline solution under the same conditions.

The animals were kept and manipulated in accordance with the rules for keeping experimental animals established by EU Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010.[10] All procedures were approved by the bioethics committee of the State Establishment “Saint Luka Lugansk State Medical University,” Lugansk, People’s Republic of Lugansk (protocol number 2 dated March 25, 2022). The animals were removed from the experiment by the decapitation method. Immediately after removal, the thyroid gland was divided into pieces of 1 mm^3 sizes, then fixed in 2.5% glutaraldehyde solution, followed by treatment in 1% osmium tetroxide, according to Palade.[11] After dehydration in increasing concentration of ethanol and absolute acetone, the material was poured with a mixture of epoxy resins (epon-araldite). Polymerization was carried out for 36 h at 60°C. Ultrathin sections were made on a UMTP-4 ultramicrotome of the Sumy PO Electron, contrasted in a solution of uranyl acetate and lead citrate according to Reynolds[12] and studied under an EM-125 electron microscope with further photography.

Results

The results of the study showed that in the animals of the control group, the thyroid gland consisted of typical follicles filled with colloids. Follicular cells are closely opposed to each other, had a predominantly cubic or columnar shape, and their apical surfaces are characterized by numerous irregularly spaced microvilli. Typical tight junctions or interdigitations are found between neighboring cells.

The nucleus of the follicular cell is predominantly oval or round in shape, located in the central or basal part of the cell, had slightly sinuous contours. Heterochromatin is located in a narrow, discontinuous rim under the nuclear membrane. The nucleolus is visualized in the peripheral part of the karyoplasm and had a different electron density. The rough endoplasmic reticulum (rER) of the follicular cell is distributed throughout the cytoplasm, and its cisterns are expanded toward the base of the cell. The cytoplasm contained single free ribosomes, numerous typical elongated mitochondria with a homogeneous, and fine-grained matrix. A moderate number of electron-dense lysosomes are distributed mainly in the apical part of cells. A noticeable zone is visualized near the nucleus, containing the Golgi complex, consisting of a group of cisterns with densely packed vacuoles and small vesicles. The apical part of the cytoplasm contained several small bubbles and typical large colloidal droplets with a substance having a similar electron density to the colloid.

The bases of the epithelial cells of the follicles are adjacent to the fenestrated capillaries, in the lumen of which electron-dense blood cells are determined.

The stroma in the thyroid gland of the control animals is moderately developed [Figure 1].

Electron microscopic examination of the thyroid gland of experimental animals revealed that the follicular cells had a cuboidal shape, less often flat. The apical surface of these cells contained a small number of low microvilli. The rER of most thyrocytes was enlarged. In general, the rER tubules contained homogeneous material, but some had abnormal electron-dense deposits. In some cases, the assembly in the tubules progressed to well-defined clusters of wavy structures of various sizes with a circular orientation, located loosely relative to each other.

In most cells, the nuclei were often irregular in shape with irregular contours, compared to the control. Heterochromatin occupied almost the entire periphery of the karyoplasm. The mitochondrial matrix was electron-dense. Lysosomes were evenly distributed in the cytoplasm. A small number

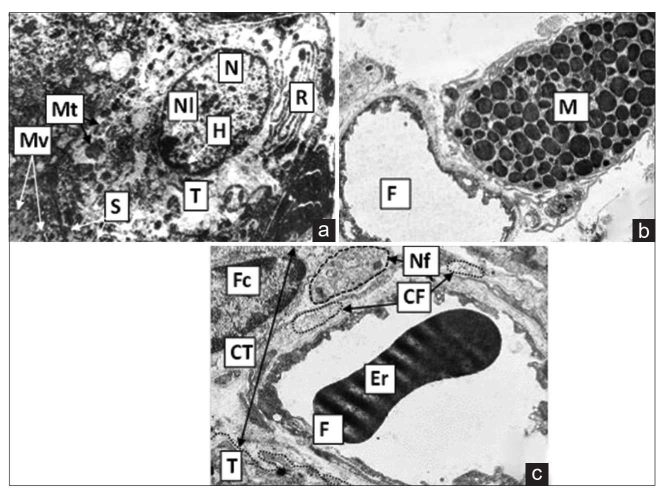

Figure 1: The piece of the thyroid gland of control mature rats ([a] piece of the thyroid gland with thyrocyte, [b] piece of thyroid gland with mast cell, [c] piece of the thyroid gland with connective tissue between follicles). T: Thyrocyte, N: Nucleus, Nl: Nucleolus, H: Heterochromatin, R: Rough endoplasmic reticulum, Mt: Mitochondria, M: Mast cell, S: Secretory granules, Mv: Microvillus, F: Fenestrated capillary, Er: Erythrocyte, CT: Connective tissue between follicles, Fc: Fibrocyte, CF: Collagen fibers (cross-section), Nf: Nerve fiber (×8000)

of small pinocytic vesicles with colloids were located in the apical part of most thyrocytes. A wide electron-light pericapillary space was observed between the basal part of the latter and fenestrated capillaries [Figures 2 and 3].

Discussion

Thus, ultramicroscopic examination showed that the thyroid gland of mature male rats contains thyrocytes of typical structure. Follicular cells delimit the colloid cavity and form the follicles. The stroma is moderately developed. A similar ultramicroscopic structure of thyroid follicular cells is confirmed by the data obtained by other researchers:

- In control, male mature outbred rats, thyrocytes of columnar shape contain interdigitations on the lateral surfaces, microvilli – on the apical surface and tight junctions on apicolateral borders. The nucleus is located in the basal part of the cell. Nucleolus of different electronic density occupies the area under the nuclear membrane. The rER is well developed, and mitochondria were randomly spread in the cytoplasm. The Golgi apparatus is located near the nucleus[13]
- In control male mature African giant rats, the thyroid gland is enclosed by a thin connective tissue capsule. The parenchyma consists of round, oval, or sometimes irregularly shaped follicles of variable sizes. The peripheral part of the thyroid gland contains large follicles lined with cuboidal or squamous follicular cells, while the central part – small- and medium-sized follicles lined with columnar or cuboidal cells. The apical surface of the thyrocyte has microvilli, apicolateral borders, tight junctions, and the base of the cell rests on the basement membrane. The nucleus was oval-shaped in the cuboidal or columnar follicular cells and flattened in squamous cells. In general, the nucleus occupies the central or basal part of the cell. The cisterns of rER and round- or rod-shaped mitochondria were distributed throughout the cytoplasm. Golgi apparatus is well developed and situated close to the nucleus[14]
- In control male mature Wistar rats, the thyrocytes contain a prominent nucleus, well developed rER, and small dense lysosomes. The Golgi complex is placed near the nucleus of the cell. The numerous long, regular microvilli projecting into the follicular lumen are situated on the apical surface of thyrocytes.[15]

In the experimental group, in comparison with the control, a small number of microvilli and small secretory granules in the apical part of thyrocytes were visualized on electronograms. Lysosomes were evenly distributed in the cytoplasm. Follicular cells were found to be cuboidal, less often flat in shape. Similar structural changes in thyrocytes were observed with exogenous administration of thyroid hormones into the body of rats, which, as shown by the study, reduced the level of endogenous thyroxine.[15] The systematic intake or high concentration of sodium benzoate in mice, reduces the synthesis of leptin by adipocytes.[16] The decrease in leptin, acting through the arcuate nucleus of the hypothalamus, leads to a decrease in the production of thyroliberin, and, consequently, reduces the production of thyrotropine.[17] The latter acts on the follicular cells of the thyroid gland and reduces the synthesis of thyroxine.

It should be noted that in the cytoplasm of the follicular cells of the thyroid gland of experimental animals, dilated cisterns of the rER are observed, in which there are single wavy structures of various sizes with a circular orientation, located loosely to each other. On the periphery of the nucleus, there are significant accumulations of heterochromatin, the mitochondrial matrix is electron-dense. The high doses of sodium benzoate cause DNA damage not only to the nuclei of rat liver epithelial cells but also to mitochondria.[18] DNA mutations can lead to impaired protein synthesis and a slowdown in its transport from the rER.[19,20] These studies can explain the changes revealed in the electronograms of the experimental group of rats.

Conclusion

In animals of the control group, the thyroid gland follicular cells had a typical structure. The 60-day administration

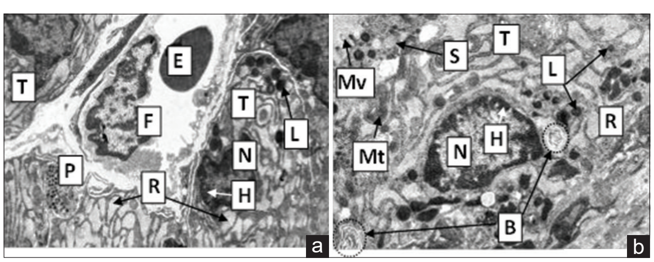

Figure 2: The piece of the thyroid gland of experimental mature rats ([a and b] pieces of thyroid gland with thyrocytes). T: Thyrocyte, N: Nucleus, H: Heterochromatin, R: Rough endoplasmic reticulum, B: Fibrous deposits in the cisterns of the rough endoplasmic reticulum, Mt: Mitochondria, L: Lysosomes, S: Secretory granules, Mv: Microvillus, F: Fenestrated capillary, E: Erythrocyte, P: Parafollicular cell (×8000)

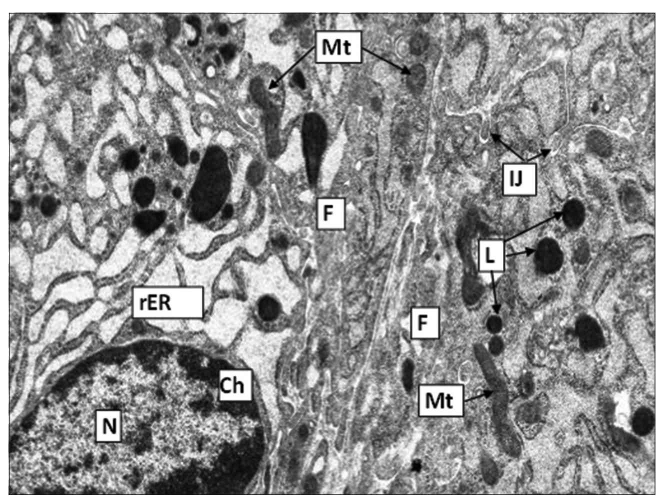

Figure: 3: The area of the thyroid gland of mature rats (experimental group). F: Follicular cells, N: Nucleus, Ch: Chromatin, rER: Rough endoplasmic reticulum, Mt: Mitochondria, L: Lysosomes, IJ: Intercellular junctions (interdigitations) (×16,000)

of sodium benzoate to rats causes adverse ultrastructural changes in thyroid follicular cells. The rER undergoes the greatest morphological changes. The above-mentioned ultrastructural changes can be explained by the direct effect of the sodium benzoate on the DNA of the nucleus and mitochondria of thyrocytes or indirectly through a decrease in the level of leptin and, as a consequence, the effect on the hypothalamic–pituitary–thyroid axis.

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Conflicts of interest

There are no conflicts of interest.

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