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N.B. Hryntsova, A.M. Romanyuk, S.S. Zaitseva, O.V. Gordienko, I.V. Khomenko, L.I. Kiptenko
Sumy State University, Medical Institute, Sumy

EFFECT OF L–TOCOPHEROL ON MORPHOLOGICAL REFORMATIONS OF RAT PINEAL GLAND UNDER THE IMPACT OF HEAVY METAL SALTS

e-mail: natalia.gnryntsova@gmail.com

The article presents the results of the study on morphological rearrangements of structural changes in the rat pineal gland under the influence of heavy metal salts and correction with L-tocopherol during 30 days. General morphological and statistical research methods were used (histological, morphometric and statistic methods). The protective effect of L-tocopherol caused in the pineal gland a number of compensatory-adaptive processes aimed at neutralizing the stressor's impact on the organ. The 30-days protective period of L-tocopherol is insufficient to completely neutralize the impact of heavy metals on the organ: the glial response was activated, vascular area increased, vascular wall permeability was impaired, edema was formed and the connective tissue component growth was taking place, indicating dysfunctional reorganization in the gland's secretory activity. The above morphological changes negatively affected the processes of hormones evacuation into the blood, the course of the general adaptation syndrome and the homeostasis restoration in the organ.

Key words: general adaptation syndrome, indolamines, hypoxia, hormones

Н.Б. Гринцова, А.М. Романюк, С.С. Зайцева, О.В. Гордієнко, І.В. Хоменко, Л.І. Кіптенко

ДІЯ L–ТОКОФЕРОЛУ НА МОРФОЛОГІЧНІ ПЕРЕБУДОВИ ЕПІФІЗА ЩУРІВ ЗА УМОВИ ВПЛИВУ СОЛЕЙ ВАЖКИХ МЕТАЛІВ

У статті представлені результати дослідження морфологічних перебудов структурних змін в епіфізі щурів під впливом солей важких металів та корекції L-токоферолом на протязі 30 днів. Використовувались загальні морфологічні та статистичні методи дослідження (гістологічні, морфометричні та статистичні). Захисна дія L-токоферолу викликала в епіфізі ряд компенсаторно-адаптаційних процесів, спрямованих на нейтралізацію впливу стресора на орган. Але, 30-денного захисного періоду L-токоферолу недостатньо, щоб повністю нейтралізувати вплив важких металів на орган: активувалася гліальна реакція, збільшувалася площа судин, погіршувалася проникність судинної стінки, формувався набряк і зростала сполучнотканинна складова частина залози, що свідчить про дисфункціональну перебудову секреторної діяльності органу. Вищевказані морфологічні зміни негативно вплинули на процеси евакуації гормонів у кров, перебіг загального адаптаційного синдрому та відновлення гомеостазу у органі.

Ключові слова: загальний адаптаційний синдром, індоламіни, гіпоксія, гормони

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The development of the pathology of individual organs and systems undoubtedly depends on adverse environmental factors. Particular attention of researchers attracts heavy metal salts. Rapid urbanization causes a significant anthropogenic and technogenic load on the environment by increasing the number of chemical elements in the air, soil, water resources, living organisms, and plants [10, 11, 15]. Environmental pollution occurs during the operation of thermal power plants, metallurgical, machine-building and chemical production, vehicle emissions [10]. The leading pollutants are heavy metals, due to their harmful effects on the health of living organisms, toxicity and the ability to accumulate in humans and animals [12]. According to the literature, heavy metals include about 40 chemical elements with an atomic mass of more than 50 units: Fe, Zn, Pb, Hg, Mn, Cu, Cr, Co, V, Mo, Cd, Sn, Ni, Bi and others [9]. It contributes to impairment of metabolic reactions, damage to membranes, reduced cell antioxidant protection, impaired protein and nuclear acids synthesis, adversely affects the physiological activity of living bodies [7, 14]. Today, an important environmental problem of some northern regions of Ukraine is the accumulation of heavy metal salts (zinc, chromium, lead, manganese, copper and iron) in the soil, water and air, which is observed in various combinations depending on the region and causes adverse effects on population's health. Such a negative impact determines the development and course of oncological pathology, disorders of the body homeostasis and morphological transformations in various tissues. The endocrine system together with the immune and nervous systems maintains homeostasis in the body. The central link, in particular the pineal gland, is involved in triggering a stress response, limiting its further development preventing adverse effects on the body [9, 12]. As of today, the various external and internal factors impact the pineal gland: antipsychotic therapy and neuroleptics [2], electromagnetic radiation [1], light and radiation in the experiment [6], fluorine [8], the degree of pineal gland calcification in the aged people [13] has been extensively studied.

The purpose of the study was to elucidate the morphological rearrangements of the structural components changes in the pineal gland of mature rats after the short-term influence of heavy metal salts and correction with L-tocopherol.

Material and methods. The experiment was performed on 12 white mature male rats weighing 200–250 g at the age of 7–8 months, which were divided into 2 groups (group 1 control and group 1 experimental). Animals of both groups were kept in normal vivarium conditions, where equal conditions of management, nutrition, proper care and natural lighting (day/night) with a constant ambient temperature (20–22°) were maintained. The animals had free access to drinking water. Animals of the experimental group were simulated microelementosis by adding to drinking water a mixture of heavy metal salts for 30 days: zinc (ZnSO4 7H2O) – 5 mg/L, copper (CuSO4 5H2O) – 1 mg/L, iron (FeSO4) – 10 mg/L, manganese (MnSO4 5H2O) – 0.1 mg/L, lead (Pb (NO3) 2) – 0.1 mg/L and chromium (K2Cr2O7) – 0.1 mg/L and received L-tocopherol corrective drug (9.1 mg/kg of 10 % oral oil solution). Dose recalculation for animals was taken into account. Selection and calculation of the drug dose was carried out based on the mean therapeutic daily dose for adults, which is 100 mg per day (30 drops of 10 % solution). The dose calculation for rats was performed taking into account the recommendations of R.S. Rybolovlev and Yu.R. Rybolovlev according to the formula: dose for rats = r × Dose for humans/R, where r is the coefficient of species endurance for rats, r=3.62, R is the coefficient of species endurance for humans, R=0.57. The selected concentration of salts in the mixture was due to the presence of similar concentrations of these salts in the soil and drinking water of some regions of Ukraine. Animals were kept and manipulated in compliance with national and international norms on bioethics. Groups of experimental animals were sacrificed after previous thiopental anesthesia (at the dose of 30–40 mg/10 g body weight) on the 30th day of the experiment (Minutes No. 8 of 17.11.2020 of the Bioethics Commission of Sumy State University). The subject of the study was the pineal gland of experimental and control animals. To study morphological changes of the pineal gland's structural components, the usual methods of microanatomical (histological) study were used. General morphological and morphometric analysis was performed using a light optical microscope "Leica DM 500" with lenses x4, x10, x40, oculars 7, 10. Photo documentation of the results was performed with a digital video camera "Leica DM IC C50 HD Camera" (Leica Microsystems, Germany, 2010). To determine the morphofunctional features of the pineal gland's pinealocytes the following morphometric parameters were used: large and small diameters of cells and cell nuclei (μm), cross-sectional area of pinealocytes and their nuclei (μm²), cytoplasm's area (μm²), nuclear-cytoplasmic ratio, pinealocytes' optic density of the nucleus and cytoplasm (RU). In order to study the ratio of pinealocytes and glial elements in the pineal gland, the absolute number of pinealocytes, astrocytic glia and gliocyto-neuronal index were determined. To determine the morphological rearrangements in the pineal gland's vascular bed, the area of the vessels (μm²) was determined. Statistical processing of the obtained data was performed by parametric method of variation statistic using the software package STATISTIKA v.10 ("StatSoft Inc.", USA). Data are presented as the mean (X) ± standard deviation (SD), using the Student's t test. The error probability of less than 5 % ($p \leq 0.05$) was considered sufficient.

Table 1

Results of the pineal gland structural components' morphometric study in sexually mature rats under the heavy metal salts impact and correction with L- tocopherol (X±CD)

Index	Groups of laboratory animals	
	Rats of the control group n=6	Rats of the experimental group, n=6
Large diameter of pinealocyte nuclei, μm	2.53±0.29	3.58±0.19***
Small diameter of pinealocyte nuclei, μm	1.34±0.04	2.23±0.29
Cross-sectional area of pinealocyte nuclei,μm ^{2}	4.45±0.13	8.53±0.07***
Large diameter of pinealocyte bodies, μm	5.26±0.14	8.70±0.06*
Small diameter of pinealocyte bodies, μm	4.12±0.07	2.63±0.02*
Cross-sectional area of pinealocyte bodies,μm	22.73±0.16	29.00±0.14
Area of the pinealocyte cytoplasm, μm ^{2}	18.28±0.58	20.47±0.91
Nuclear cytoplasmic ratio	1:0.24±0.18	1:0.42±0.14
Optical density of the nucleus, RU	114.09±1.19	110.76±0.52
Optical density of cytoplasm, RU	154.06±0.11	110.76±0.55***
Vessels area, μm ^{2}	58.58±0.42	266.56±0.98***
Absolute number of pinealocytes	112.67±1.93	90.00±1.41***
Absolute number of astrocytic glia cells	35.97±1.95	99.83±29.68***
Glyocyto-neuronal index	0.34±0.44	1.09±1.05

Note**:** Reliable compared to the control: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Results of the study and their discussion. 30 days of exposure a complex of heavy metal salts on the body caused negative morphological changes of nonspecific polymorphic nature in the pineal gland of mature rats, which were expressed in the increased vascular area, active glial response, hypertrophy of pinealocyte nuclei and increase of their optical density. Cytological signs of most pinealocytes indicated the predominance of indolamine synthesis over polypeptides. The main pathogenetic mechanisms of the influence of heavy metal salts on the organ have been established: change in the vascular lumen area, blood rheological properties impairment, tissue hypoxia, nucleus hypertrophy and change in their optical density.

The protective effect of vitamin E (α-tocopherol acetate) on the pineal gland's structures at 30 days of exposure to a combination of heavy metal salts was studied. In this case, the pineal gland of the experimental animals retained its anatomical structure, had an oval shape, but the linear parameters changed (table 1).

The length of the gland decreased by 9.44 % (1.074±0.04 mm; $p<0.05$, $t=2,4$), the width was reliably reduced by 24.4 % (0.689±0.072 mm; $p\leq0.05$, $t=2.84$) compared to the intact animals, the capsule thickening by 4.4 times ($p<0.001$, $t = 5.72$).

The value of the gland's fibrous connective tissue component was increased (fig. 1).

A reactive compensatory response of the gland's stromal component was observed as the increased distinctiveness of the trabecular connective tissue component.

In the pineal gland's vascular bed, changes in the vascular blood filling (initial stages of venous plethora formation), increased permeability of the vascular wall and the initial signs of the blood rheological properties impairment were determined (fig. 2).

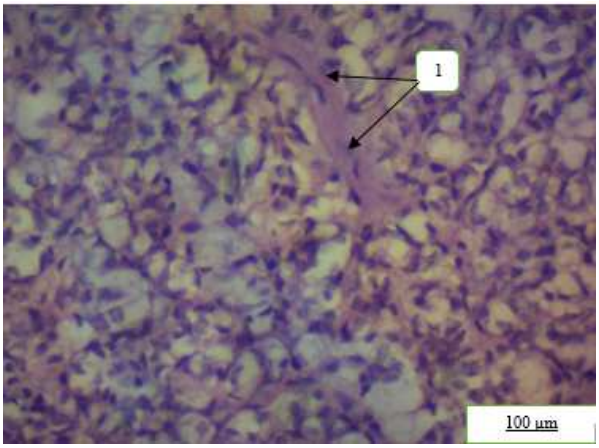

Fig. 1. Morphological rearrangements of the pineal gland's structural components under the condition of 30 days exposure to heavy metal salts and correction with α-tocopherol acetate: 1 – increase in the severity of the gland's trabecular connective tissue component. Hematoxylin-eosin staining

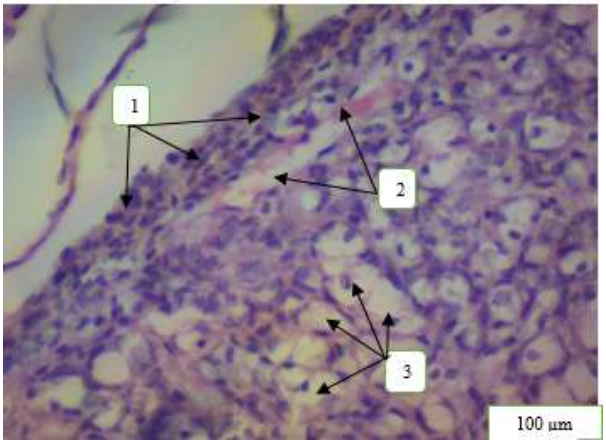

Fig. 2. Morphological rearrangements of the pineal gland's structural components under the condition of 30-days exposure to heavy metal salts and correction with α-tocopherol acetate: 1 – reactive glial response; 2 – aggregation and sludge of erythrocytes; 3 – pinealocytes with signs of vacuolation and polypeptide production. Hematoxylin-eosin staining

Vessels of the microcirculatory bed in the intertrabecular spaces were dilated, full-blooded. The degree of total gland's vascularization compared to the control and group I animals, increased without the protector. Large venules in the pineal gland parenchyma had a thickened vascular wall with an increased of permeability degree and signs of collagenization. Active pericellular edema was formed around such vessels.

In one of the fields of view, perivascular connective tissue vegetation was determined, probably at the site of lost pinealocytes and reactive glial response. The nuclei of vascular endotheliocytes were well contoured, were hyperchromic. In vessels the initial stages of blood rheological properties disturbance with formation of a stasis, aggregation of blood cellular elements with capillary endothelium, a sludge phenomenon were visualized. In the subcapsular zone, the most pronounced circulatory disorders were observed in the form of hemorrhages of various size and shape and diffuse permeation with erythrocytes and plasma parenchyma of the gland. The area of vascular lumen significantly increased by 4.5 times ($p<0.0001$) compared to the control animals.

In the peripheral areas of the gland, subcapsularly, around the vessels and diffusely in the parenchyma, an intense reactive proliferative response of neuroglia was retained in response to the damaging agent. Thus, the absolute number of pinealocytes decreased compared to the control animals by 20 % ($p<0.0001$), and the absolute number of astrocytic glia cells, in contrast, increased by 2.8 times

($p<0.0001$). The glyocyto-neuronal index increased and exceeded the indices of control animals by 3.2 times ($p>0.05$).

In the gland there was a change in the type of secretory activity. The gland's parenchyma in animals of experimental group was dominated by peptide-producing pinealocytes. There were changes in the structure of pinealocytes. Pinealocytes had a cytoplasm overstretched by a large light optically transparent vacuole. At the same time, the index of pinealocyte body's large diameter was increased by 65.4 % ($p>0.05$), and the index of small diameter, on the contrary, decreased by 36.2 % ($p>0.05$) compared to the control animals. The cross-sectional area of the cytoplasm and pinealocyte bodies grew by 12 % ($p>0.05$) and 27.6 % ($p<0.0001$), respectively, compared to the control animals. The cell nuclei were hyperchromic, homogeneous, elongated, and oval shaped, rich in euchromatin, slightly increased in size. A small number of pinealocytes showed signs of indolamine synthesis. The pineal gland's parenchyma had a small number of small, oval-shaped cysts that were filled with light transparent fluid. Thus, the pinealocytes nuclei large diameter index grew by 41.5 % ($p<0.0001$), and the index of their small diameter – by 66.4 % ($p>0.05$) compared to the indices of the control animals. The cross-sectional area of pinealocyte nuclei was increased by 1.9 times ($p<0.0001$) compared to the control animals. The optical density of the nuclei and cytoplasm in pinealocytes decreased by 3 % ($p>0.05$) and 39 % ($p<0.001$), respectively, compared to the control animals, which probably indicated an improvement in the synthetic activity of pinealocytes when using the L-tocopherol corrector drug. The nuclear-cytoplasmic ratio increased to 1:0.42±0.14 compared to the control animals.

The protective effect of α-tocopherol acetate on the state of the pineal gland's structural components in mature rats at 30-days exposure to a combination of heavy metal salts was determined. At the same time, the linear parameters of the organ remained significantly lower than those of the control animals. The protective effect of α-tocopherol acetate was, first of all, to activate the reactive glial response, to increase the pronouncement of the gland's stromal component, to improve the secretory activity of pinealocytes and to change the type of their secretion. A reactive compensatory response of the gland's stromal component was observed. Thus, in contrast to animals that received only salts of heavy metals, in the pineal gland of animals with tocopherol correction significantly increased not only the thickness of the capsule, but there was an increase in the pronouncement of the trabecular connective tissue component, as well as vascular collagenization. This can be explained by the activation of fibroblasts under the action of hypoxia, because the effect of α-tocopherol acetate at this term of the experiment is still insufficient to fully restore the condition of the vascular wall and the rheological properties of blood. This, in its turn, prevented the complete evacuation of hormones through the vascular wall into the blood and affected the course of the general adaptation syndrome. The increase in the permeability of the vascular wall led to the development of perivascular edema in the gland, and the formation of hemorrhages in the peripheral areas of the organ. The vascular area was significantly and reliably increased compared to the control animals, group I animals and continued to affect the correlation between the vascular area and the nucleus area, the number of astrocytes and pinealocytes. However, the correlations between the area of the cytoplasm and the area of the vessels, the area of the vessels and the optical density of the nucleus did not differ from those of group I animals. On the part of pinealocytes, there was an improvement in their secretory activity and a change in the type of secretion. Pinealocytes with cytological features of polypeptide production predominated in the specimens [1]. The condition of the nucleus and nucleoreticulum was significantly improved, the area of the nucleus decreased compared to the indices of animals, receiving only heavy metal salts, the optical density of the nuclei was almost equal to that of the control animals, which can be considered a positive protective effect of α-tocopherol acetate on the cell status. The presence of a large number of vacuolated cells and cysts in the parenchyma of the pineal gland indicates the delay of the hormones' evacuation into the vascular bed as a result of heavy metal salts impact on the plasmalemma of pinealocytes, the vascular wall and disruption of the hormones diffusion into the blood. The neuroglia reaction in response to the damaging agent is also activated, especially in the peripheral subcapsular areas of the gland. The formed perivascular astroglial complexes according to [5] may indirectly indicate more intense processes of pineal cell apoptosis in these animals, as evidenced by a reliable decrease in the number of pinealocytes compared to the control animals' indices. In addition, the increase in the number of glial elements in the pineal gland certainly have a certain compensatory-adaptive value, especially in the transfer of RNA, amino acids, growth factors to pinealocytes and controlling water-ion homeostasis in the gland [3,4]. According to the authors, such proliferates perform a barrier function, preventing the penetration of heavy metals into the parenchyma of the gland. It is impossible to overestimate the contribution of astrocytes in the protection of the gland's parenchyma from oxidative stress through the synthesis of

hydrogen sulfide (H_2S). This gas gliotransmitter has synaptic modulator and neuroprotective properties, protecting the pineal parenchyma from oxidative stress [4].

Conclusion

The protective effect of L-tocopherol caused a number of compensatory-adaptive processes in the pineal gland aimed at neutralizing the effects of stressors on the organ. The condition of the pinealocytes' nuclear apparatus significantly improved, the size of nuclei and the index of their optical density decreased, which indicated a significant improvement in the synthetic activity of pinealocytes. There was a change in the secretion of the gland to that of peptide-producing. However, the 30-days protective period of L-tocopherol is insufficient to completely neutralize the impact of the heavy metal on the organ: the glial response activated, the vascular area increased, vascular wall's permeability impaired, edema was formed and the connective tissue component grew, indicating dysfunctional reorganization of the gland's secretory activity. The above morphological changes in the pineal gland's structural components negatively affect the processes of hormones evacuation into the blood, the course of the general adaptation syndrome and homeostasis restoration in the organ. The studies of the pineal gland, of course, expand the range of knowledge about the participation of this central neuroendocrine system's organ in the body's adaptive response to the combination of heavy metal salts and encourage researchers to seek for new drugs to correct morphological changes in the organ.

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