Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-5 Year 2023** Page **301:305***

Histochemical Indicators of The Adrenal Glass Under Acute Exposure to Magnesium Chlorate

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 13 Oct 2023

Abstract

Acute administration of defoliant leads to structural, hemodynamic disorders and dystrophic changes in the cells of the cortex and medulla of the organ. The relative weight of the adrenal glands was significantly increased in comparison with the control. Expansion of the relative width of the adrenal cortex and its zones is noted. The volume of nuclei of cortical and chromaffin cells was significantly increased in comparison with the control. The vascular reaction manifests itself not only in hyperemia, but also in local hemorrhages. Determined by karyopyknosis in cortical cells, vacuolization of their cytoplasm. After a single injection of the pesticide, there is a decrease in the synthetic processes of the secretory cycle in the cells of the cortex zones. A decrease in the amount of lipids and ascorbic acid indicate an increase in the phase of hormone release from cortical cells. In the medulla, the content of N cells is increased, the secretory activity of most chromaffin cells is moderately expressed.

Keywords: *Acute Poisoning, Magnesium Chlorate, Adrenal Glands, Gistochemical Changes.*

1. Introduction

Currently, the chemicalization of agriculture creates new environmental factors that need to be taken into account (Rogozin M.Yu., 2018) [7]. To date, the adverse effects of many pesticides on the body have been proven, which leads to the development of pathological conditions in it (Aksenov V.A. et al., 2016) [1]. In recent years, the state of the organs of the digestive system, immune and reproductive systems under the influence of various exogenous factors has been studied again (Lencher O.S., 2016) [6].

At the same time, along with new pesticides, magnesium chlorate is still used in agriculture as a defoliant and desiccant (Blinova S.A. et al., 2021) [2]. The literature does not contain sufficiently complete information about the state of adaptive reactions in the adrenal glands after exposure to pesticides, including magnesium chlorate. All this justifies the need for a detailed study of morphofunctional changes in the adrenal glands during single and repeated exposure of the body to various doses of magnesium chlorate, which is very relevant in its significance.

The purpose of the study was to establish histochemical changes in the adrenal glands during acute exposure to magnesium chlorate.

2. Materials And Methods

The study was carried out on 18 adult outbred male rats, weighing 150-200 g. Magnesium chlorate at a dose of 410 mg/kg body weight of the animal was administered intragastrically, in the form of a 4.1% solution, on an empty stomach. The pesticide was dissolved in distilled water. The indicated dose corresponds to 1/10 LD_{50}. A regular syringe with a metal probe was placed deep into the oral cavity of the rats and the drug was slowly injected. The oral route of administration of magnesium chlorate was

chosen taking into account the fact that, according to a number of authors, in 85-90% of cases pesticides enter the body with food and water. The dose of magnesium chlorate equal to LD_{50} (average lethal dose) for rats is 4.1 g/kg body weight. At the same time, a dose of magnesium chlorate equal to 1/100 LD_{50} (41 mg/kg) for rats corresponds to the threshold; it is this dose that the human and mammalian body most often encounters in areas of intensive pesticide use, so in practical terms its use was important. The animals of the first series (6 rats) were intact. Animals of the second series (6 rats) served as controls. Under similar conditions to rats exposed to magnesium chlorate, they were given distilled water. The state of the adrenal glands was studied in 6 rats that received a single dose of distilled water. In the third series, 6 rats were subjected to a single exposure to magnesium chlorate at a dose of 1/10 LD_{50}.

All animals (intact, control and experimental) were kept in the same vivarium conditions. No animal mortality was observed. Before the start of the experiment (for one month) and during the entire period of administration of the drug, the rats were constantly monitored: the general condition was noted, the weight, stool and mobility of the animals were monitored. The animals were weighed before the start of the experiment and after its completion. Rats receiving magnesium chlorate, as well as control and intact rats, were killed simultaneously by instant decapitation using a specially constructed guillotine. The adrenal glands were removed immediately after the animal was slaughtered and weighed on a torsion scale.

To assess possible reactive and compensatory changes in the state of the adrenal glands after administration of magnesium chlorate, we used histological and histochemical research methods. After weighing the adrenal glands along the conventional transverse axis of the organ, they were divided in half so that the condition of the cortex and medulla could be assessed.

To fix the adrenal glands, a 12% solution of neutral formalin, Carnoy's fluid, Becker's fluid, a 10% solution of silver nitrate and Sevka's fluid were used. After appropriate processing of the material according to the selected methods, it was passed through alcohols of increasing concentration and embedded in paraffin. Sections with a thickness of 5-7 mkm were prepared from paraffin blocks. The sections were stained with hematoxylin-eosin, picrofuchsin according to Van Gieson, and impregnated using the Foote method. The PAS reaction was also carried out to detect glycogen with amylase control, the Brachet reaction to detect RNA with ribonuclease control, Sudan black staining to determine lipids, the detection of ascorbic acid using the Giroux-Leblond method and the Sevka reaction (a type of chromaffin reaction).

The selected methods make it possible to assess the condition of the zones of the cortex and medulla of the adrenal glands, their cellular and fibrous structures, the localization and spread of the pathological process in the adrenal glands. In addition, histochemical methods make it possible to present the morphofunctional characteristics of adrenocorticocytes and chromaffin cells of the organ.

The histochemical methods we used make it possible to judge not only the functional state of the adrenal gland, but also to determine the violation of the stages of the secretory cycle in its glandular cells. Thus, the RNA content is an indicator of the intensity of the synthesis of steroidogenesis enzymes. Glycogen is necessary to initiate the synthesis of adrenal hormones. Lipids are the material for hormone formation in the cells of the gland cortex. Ascorbic acid most of all reflects the stage of hormone release from cortical cells.

A quantitative assessment of the results obtained was carried out:

1. The relative weight of the adrenal glands was calculated in mg of absolute weight per 100 g of animal weight (mg%).

2. The relative width of the cortex and its zones was calculated in microns of the width of the cortex and zones per gram of body weight.

3. The volume of the nuclei of 100 cells of each zone of the cortex and medulla was measured using the formula of A. Arnold [2].

4. Counting ascorbic acid granules in 50 cells of each cortex zone.

5. The content of RNA, glycogen and lipids was determined using a five-point rating scale, followed by determination of group average values (semi-quantitative method of analysis). 0 - absence of substance; 1 - very little; 2 - not enough; 3 - moderate amount; 4 - a lot; 5 – is quite a lot [2].

6. In order to objectify the results of the scoring, a cytophotometric study of the content of RNA, glycogen and lipids was carried out in histological preparations of the adrenal glands of intact, control and animals with a single injection of magnesium chlorate, and with repeated administration on days 3 and 90. The study was carried out on a laboratory cytophotometer "Carlzeiss" in a monochromatic beam with a wavelength of 546 nm, using a mirror diaphragm of size d = 0.1 mm, recording time 1 s [2].

Statistical processing of the resulting digital material was carried out with the calculation of average errors for arithmetic means (M ± m). The degree of significance of the difference was calculated based on the definition of Student's t - test. Then, using the student's t-test table, the probability (P) of a possible error was determined. Differences between control and experiment were considered significant when $0.001 \leq P \leq 0.05$.

3. Results and Discussion

This series provides data on the study of the morphofunctional activity of the adrenal glands of rats under a single exposure to magnesium chlorate at a dose of 1/10 LD_{50} (acute poisoning). The studies were carried out on 6 rats. In rats of this series, there was an increase in the relative weight of the adrenal glands compared to the control, which amounted to 0.155 ± 0.0022 mg per 100 g of animal body weight ($P < 0.001$).

Histological structure of the cortex and medulla of the adrenal glands. Histological examination of the adrenal glands reveals thickening of their capsule as a result of edema. The surface of the organ forms many protrusions of various sizes. The cytoplasm of some cells is clumpy. Argyrophilic fibers are varied in various ways, fragmented, many of them even to the point of lysis, and the rest are thickened. Zonal differentiation of the cortical zones is somewhat impaired. In the zona glomerulosa, the arrangement of cells is disturbed, as a result of which the glomeruli are poorly distinguishable. Some cells in this zone are subject to hydropic degeneration. In this case, adrenocorticocytes are flattened with elongated nuclei and oriented parallel to the capsule, with symptoms of karyopyknosis. Focal hemorrhages are noted. Fragmentation and focal lysis of argyrophilic fibers are observed.

Morphometric indicators and histochemical picture of the functional state of the adrenal cortex. A morphometric study reveals a significant increase in the relative width of the adrenal cortex compared to the control. The relative width of the zona glomerulosa, zona fasciculata and reticularis is also significantly greater than in control rats (Table 1). The volume of cell nuclei in the glomerular, fascicular and reticular zones of the cortex was significantly increased compared to the control (Table 1).

A single injection of magnesium chlorate at a dose of 1/10 LD_{50} causes sharp changes in the quantitative content of the analytes in the bark. In the zona glomerulosa of the adrenal glands, there is a decrease in the content of RNA, glycogen, lipids and ascorbic acid. In this case, high pyroninophilia of subcapsular cells is noted. In the zona fasciculata of the organ, there is also a smaller amount of RNA, glycogen, lipids and ascorbic acid than in the control. In the reticular zone, the content of histochemical substances - RNA, glycogen, lipids and ascorbic acid - was reduced compared to the control.

Table 1. Morphometric parameters of the adrenal glands in rats receiving a single dose of magnesium chlorate at a dose of 1/10 LD_{50}

An object	Adrenal	Glomerular	Beam	Reticular	Medulla
Relative naya width of the cortex and its zones (mcm	512,3±9,34*	42,46± 2,63***	347,23± 4,32*	122,59± 3,4*	-
Core volume (mcm3)	-	64,63±2,7*	86,69±	66,25±	101,54±

Note: P – indicator of significance of differences compared to the control $0.001 \leq P \leq 0.05$ (*–P<0.001; ** – P<0.01; *** – P<0.05).

According to the results of cytophotometry, the content of RNA, glycogen and lipids in all zones of the cortex is significantly lower than in the control (Table 2), which confirms the results of a visual assessment of the amount of histochemically detected substances.

State of chromaffin cells of the adrenal glands. In the chromaffin cells of the medulla, the nuclear volume significantly exceeds the control level ($P < 0.01$) (Table 1). In the adrenal medulla of rats exposed to a single dose of magnesium chlorate at a dose of 1/10 LD_{50}, there is an increase in the number of norepinephrine-containing cells compared to the control, and they account for approximately 50% of the total number of cells. In the cytoplasm of norepinephrine- and adrenaline-containing cells, small vacuoles and single large vacuoles are observed, which indicates a moderate secretory activity of these cells. Moreover, it is more pronounced in norepinephrine-containing cells than in adrenaline-containing cells.

Table 2. Content of histochemical substances according to data cytophotometry in the adrenal cortex in rats treated once magnesium chlorate at a dose of 1/10 LD50

Histochemical	Glomerular zone	Beam zone	Reticular zone
RNA	23,8±0,39 *	20,5±0,37 *	21,86±0,35 *
Glycogen	17,49±0,36 *	15,23±0,34 *	18,76±0,35 *
Lipids	16,69±0,36 *	13,2±0,38 *	13,97±0,37 *

Note: P – indicator of significance of differences compared to the control $0.001 \leq P \leq 0.05$ (*–P<0.001; ** – P<0.01; *** – P<0.05).

Thus, the data obtained on the morphofunctional state of the adrenal glands of rats subjected to a single exposure to magnesium chlorate at a dose of 1/10 LD_{50} differ significantly from those in control animals. As the results of morphological, morphometric and histochemical studies show, a single administration of a defoliant leads to structural, hemodynamic disturbances and dystrophic changes in the cells of the cortical and medulla of the organ. Changes in hemodynamics and the structure of blood vessels of internal organs under the influence of exogenous factors have been noted by a number of researchers (Gasanov A.G., 2009; Koko et al., 2004) [4,9].

The relative weight of the adrenal glands was significantly increased compared to the control. There is an expansion of the relative width of the adrenal cortex and its zones. The volume of nuclei of cortical and chromaffin cells was significantly increased compared to the control.

The vascular reaction manifests itself not only in hyperemia, but also in local hemorrhages. Karyopyknosis is detected in cortical cells, vacuolization of their cytoplasm. After a single administration of the pesticide, there is a decrease in the synthetic processes of the secretory cycle in the cells of the cortical zones. A decrease in the amount of lipids and ascorbic acid indicates an increase in the phase of hormone release from cortical cells. Disruption of the secretory cycle in glandular cells has been described under various influences (Kostrova O.O. et al., 2016; Odo R.I. et al., 2019) [5,10].

In the medulla, the content of N-cells is increased, the secretory activity of most chromaffin cells is moderate. In the adrenal glands, the development of adaptive reactions is observed, which is manifested by the presence of protrusions on the surface of the organ, as well as an increase in the relative weight of the organ, an expansion of the relative width of the cortex and its zones, and an increase in the volume of cell nuclei in all layers of the organ. In addition, high pyroninophilia of the cells of the subcapsular layer is observed. Most authors, when assessing the effect of various damaging factors on the adrenal glands, usually limit themselves to assessing one of the parameters (Volkova N.I. et al., 2018; Gannouni N. et al., 2014) [3,8].

4. Conclusion

Thus, the totality of these changes indicates that with a single action of the pesticide at a dose of 1/10 LD50, a sharp increase in the morphofunctional activity of the organ occurs along with the development of reactive-destructive changes in it against the background of disruption of synthetic processes in cells. After a single application of the pesticide, the secretory activity of chromaffin cells in the medulla increases, and the number of norepinephrine-containing cells in it increases. There is a sharp increase in the adaptive reactions of the adrenal glands.

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