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Morphology of Endocrine Organs in Chronic Endogenous Intoxication

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Experimental study of the effects of endogenous toxic compounds on endocrine organs showed that the pathomorphological changes were caused by the cytopathic effects of endogenous toxic compounds involving the development of stereotypical reactions to injury. The severity of these reactions depended on the initial functional status of the gland and its involvement in systemic mechanisms of adaptation to the toxic process.

Key Words: *chronic endotoxemia; endogenous intoxication; endocrinopathy; hormonal dysregulation*

Chronic endotoxemia (ET) is a serious problem of modern clinical practice. Due to adequate therapy, many chronic diseases are not associated with obvious manifestations of ET, but eventually its latent course forms a complex of polyorgan disease determining thanatogenesis.

The patho- and morphogenesis of chronic ET are sufficiently well studied. Uncontrolled transport of endogenous toxic compounds causing secondary lesions of target cells, organs, and tissues underlie the development of this condition [3,5,10-12]. The regularities of morphofunctional changes in different compartments of the autonomic nervous system and their role in the development of visceral diseases in chronic ET have been evaluated [2,9].

However, disorders of hormonal regulation received little attention. The morphological substrates of this dysregulation are organs of the endocrine system producing hormones and hormone target organs [7]. Morphofunctional studies of the endocrine organs in chronic ET and analysis of endogenous intoxication markers and of structural and functional changes in the chronic ET target organs are therefore an important problem.

We studied the regularities of morphological changes in the endocrine system organs in chronic ET and the contribution of hormonal dysregulation developing as a result of these shifts to the patho- and morphogenesis of this condition.

MATERIALS AND METHODS

The study was carried out on male and female outbred albino rats (n=141). Chronic ET with predominant involvement of the liver in experimental series I (n=60) was induced by repeated injections of low dose bacterial LPS and tetrachloromethane (TCM) [6]. The animals received TCM in a dose of 0.5 ml/kg with cheese and butter paste 6 times a week on an empty stomach. On day 6, this procedure was supplemented by intraperitoneal injection of LPS (0.2 μg/kg). Chronic ET with predominant involvement of the kidneys (experimental series II; n=60) was induced

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by daily intraperitoneal injections of 4% nephrotoxic antibiotic gentamicin (20 mg/kg). On day 6, similarly as in series I, this procedure was supplemented by intraperitoneal LPS (0.2 μg/kg). The animals were sacrificed on days 30, 60, and 90. Control group (n=21) consisted of intact rats.

Toxic hepatitis and nephropathy with hepatic and renal failure are reproduced in these models by day 30. By day 90, more pronounced disorders are reproduced: formation of cirrhosis of the liver and nephrosclerosis [7].

Chronic ET was morphologically verified by signs of progressive hepatic and renal disease with development of the compensatory processes in these organs: chronic hepatitis/hepatofibrosis and dysmetabolic nephropathy/nephrosclerosis.

Endogenous intoxication (EI) was verified by biochemical tests. Plasma levels of medium molecular weight substances, oligopeptides, and the concentration of MDA (lipid free radical oxidation product) were measured. The results confirmed the development of chronic EI in all cases (Table 1).

The material for pathomorphological studies of the endocrine organs (hypothalamic paraventricular nucleus, pituitary, thyroid gland, adrenals, ovaries/testes, pancreas) was prepared routinely [8,4].

Videotest-Morpho PC complex was used for direct morphometric studies. Numerical density and volume fraction (VF) of individual structures, sizes and their proportions were evaluated [1]. The severity of endocrinocyte injury was evaluated by the proportion of unchanged cells to endocrinocytes in a state of functional hypersecretion, extreme exhaustion, injury, or necrosis.

Immunohistochemical study was carried out using kits of antibodies to TRAIL (clone 27B12), caspase-3 (clone (JHM62), and endothelial nitroxide synthase (NOS-3, clone RN5, Novocastra Laboratories Ltd). The results were visualized by indirect immunoperoxidase method. By the degree of expression the cells were referred to one of the following classes: negative, weakly positive, positive, and hyperexpressive; the percentage of each class was evaluated.

RESULTS

Neurons with vacuolated cytoplasm predominated in the hypothalamic paraventricular nucleus. The nuclei of these neurons were often deformed and shifted towards the cell periphery; some of them had no nucleoli. Solitary ghost cells were detected among retained neurons. The VF of neuron decreased significantly with ET progress, this decrease being more pronounced in the model with predominant involvement of the liver. On day 90, the mean size of, neurons decreased (by 20% in both models). These changes were paralleled by an increase of the microglia/neuon coefficient.

Morphometry showed neurons of four types. Type I neurons were referred to intact. Type II cells corresponded to neurons in a state of functional stimulation. Type III cells exhibited signs of exhaustion. Type IV neurons were damaged and histologically looked coarsely modified and ghost cells. As ET progressed, the percentage of intact neurons decreased, as well as the counts of cells with functional hyperactivity, while the counts of exhausted cells increased. On day 90 of the experiment, 32% neurons had low functional activity, 10% neurons were damaged. Our data were in line with previous data on high sensitivity of the paraventricular nucleus to exogenous destructive factors, which was primarily due to functional loading of the nucleus and its initially poor microglia presentation.

Basophils with clarified cytoplasm predominated in the adenohypophysis, in some cases with pyknotic nuclei. Swelling and vacuolar degeneration were found in some adenocytes. Morphometry showed an increase (1.7 times) of acidophil VF in comparison with the values in control rats, while basophil VF decreased 2.1 times. These facts did not indicate greater sensitivity of basophilic adenocytes to toxins. We regarded it as functional restructuring of the adenohypophysis. According to immunohistochemical studies, by day 90 the count of TRAIL-positive adenocytes increased 7-fold and caspase-3-positive cells by 16.2 times. Increasing expression of TNF-dependent apoptosis markers with the progress of chronic ET persuasively proved the involvement of this apoptosis mechanism in the studied pathological process. By the end of the experiment, the percentage of endotheliocytes hyperexpressing endothelial NOS increased significantly (7.3 times).

The main morphological changes in the adrenals in chronic ET were endocrinocyte atrophy and compensatory stromal proliferation. Irrespective of the experimental model, the greatest changes were found at the interface of the cortical matter and the medulla. An increase in TRAIL and caspase-3 expression was found in the adrenals starting from day 30 of the experiment and reached the peak on day 90 of chronic ET. Immunohistochemical evaluation of endothelial NOS in the adrenals showed that the volume density of immunopositive cells was 10.1 times higher in chronic ET than in the control. The location of clusters of immunopositive cells coincided with sites of maximum damage and stromal proliferation.

Mosaic disorders of the organ histoarchitecture were found in the thyroid. The structure of the thyroid during ET progress underwent phasic courses and these changes were similar in different models. The gland retained the normoplastic structure during the

TABLE 1. Biochemical Characteristics of Chronic ET in Rats with Predominant Involvement of the Liver (*M*±*m*)

Parameter	Control group	Day of experiment		
		30	60	90
MMWS, arb. units	0.16±0.02	0.43±0.05*	0.49±0.05*	0.51±0.06*
Oligopeptides, mg/liter	115.5±13.8	268.5±29.8*	289.4±30.5*	291.0±36.4*
MDA, mmol/liter	5.33±0.43	11.40±0.94*	10.53±1.25*	11.08±1.35*

Note. *$p<0.05$ compared to control group. MMWS: medium-molecular-weight substances.

early periods of chronic ET. As ET progressed, the thyroid tissue was remodeled by the macrofollicular type with the formation of scanty nests with microfollicular transformation and stromal replacement of the volume of lost parenchyma. These changes manifested in reduced height and volume of the follicular epithelium, increase in VF of the colloid against the background of increased levels of the peripheral thyroid hormones.

Immunohistochemical study on day 30 showed a 5-fold increase in the incidence of TRAIL expression. By the end of the experiment, immunopositive cells formed a complete thin layer along the follicle periphery with predominant location of the ligand on the apical surface of thyrocytes. This was paralleled by an increase in caspase-3 expression.

The percentage of endothelial cells hyperexpressing NOS in the thyroid in chronic ET increased throughout the experiment: 5.1 times (by day 90) in rats with predominant involvement of the liver and 3.7 times in rats with predominant involvement of the kidneys.

Edema of the interlobular septae with moderate lymphocytic infiltration and focal hemorrhages in fatty tissue along the organ periphery was found in pancreatic islets on day 30 of ET. By day 60, sites with periductal and periacinar sclerosis were found in the gland parenchyma. Some islets were completely sclerosed, hyperchromatic nuclei were found in the majority of endocrinocytes. The expression of NOS-3 in the pancreatic islet vessels increased from 3 to 23% by day 90 of chronic ET. Studies of TRAIL expression during the early periods of the experiment showed staining of solitary endocrinocytes and acinar cells (4%). By day 90, the content of immunopositive insulocytes increased to 29%; the cells were located diffusely on the islet territory. At later terms, more and more numerous caspase-positive cells appeared.

Hence, mosaic combination of sites of injury, death, degeneration, and hypertrophy of endocrinocytes, vascular reactions, and stromal proliferation were the key pathomorphological changes in chronic ET. The degree of endocrinocyte involvement in chronic ET and the patterns of compensatory processes in a gland depended on its initial functional status, involvement in systemic mechanisms of adaptation to the toxic process under conditions of developing total trend to subcompensated hypofunction and stromal proliferation with partial replacement of the volume of lost endocrinocytes.

It is obvious that chronic ET, similarly as all syndromes progressing for a long time, forms under conditions of neuroimmunoendocrine deregulation, which also contributes to ET development. Long EI leads to the development of hormonal dysregulation characterized by disorders in the hormonal profile, dissociation of intra-axial connections, progressive restructuring of the endocrine system organs.

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