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Sex Hormone Profile and Morphological Changes in the Ovaries in Chronic Endotoxicosis

A. G. Denisov, S. A. Kalashnikova, A. I. Shchegolev*, and V. V. Novochadov

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Morphological changes in the ovaries and levels of sex hormones were studied in rats with chronic endotoxicosis. Long-term endogenous intoxication was associated with the development of sclerocystic ovary syndrome paralleled by hormone imbalance.

Key Words: *chronic endotoxicosis; sex hormones; ovary; sclerocystic ovary syndrome*

Long-term intoxication is associated with excessive release of oocytes from the pool of silent cells and subsequent development of preterm ovarian insufficiency. By the present time, many factors of toxic origin associated with early menopause were detected [6].

Direct realization of organ transformation under the effect of chronic exposure to toxic compounds (including some endogenous factors) involves the oocytes, granular cells, thecal cells, and stromal elements (fibroblasts, monocytes, vascular network). Speaking about the compensatory and adaptive characteristics of the ovaries, we have to emphasize that the final stages of ovo- and folliculogenesis (preovulatory growth and ovulation) are most sensitive to various destructive agents [1,2,7,9]. However, the data on the morphology and function of the ovaries in chronic endotoxicosis (CET) are scanty and contradictory [3,8,10].

We studied the profile of sex hormones and the type of structural changes in the ovaries of animals exposed to various toxins in experimental CET.

Laboratory of Basic Studies, Volgograd Research Center, Russian Academy of Medical Sciences and Administration of the Volgograd Region; *A. V. Vishnevsky Institute of Surgery, Moscow, Russia. ***Address for correspondence:*** kalashnikova-sa@yandex.ru. S. A. Kalashnikova

MATERIALS AND METHODS

Experiments were carried out on 50 female Wistar (185±12 g) kept under standard vivarium conditions on standard diets with free access to water and at standard day:night regimen. Synchronization of the estrus and formation of multiestrus were induced by two intraperitoneal injections of progesterone (10 μg/kg) 2 days before the experiment.

Chronic endotoxemia with predominant involvement of the liver (group 1, n=20) was induced by repeated injections of *S. typhimurium* LPS (Sigma) in a low dose of 0.2 mg/kg in combination with tetrachloromethane (0.5 ml/kg). This treatment protocol leads to the formation of toxic hepatitis and tissue fibrosis with hepatic insufficiency by day 30; by day 90 more pronounced disorders associated with the formation of cirrhosis of the liver are reproduced [5].

Chronic endotoxemia with predominant involvement of the kidneys (group 2; n=20) was induced by daily intraperitoneal injections of gentamicin (20 mg/kg) and LPS (0.2 mg/kg) once a week. The development of CET was verified by measurements of the serum biochemical markers of endogenous intoxication: medium-molecular-weight substances, oligopeptides, and MDA.

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The severity of organ involvement was evaluated by morphometric parameters of the liver (volume percent of hepatocytes, size of Kupffer cell nuclei, volume percent of connective tissue) and kidneys (urinary space, tubular index, volume percent of the interstitium, number of sclerosed glomeruli).

Changes in the kidneys by the end of the experiment corresponded to dysmetabolic nephropathy with nephrosclerosis formation [4].

The animals were sacrificed on days 30, 60, and 90 of the experiment under Nembutal narcosis (100 mg/kg). Intact rats (n=10) served as the control. All experimental manipulations were carried out in accordance with Regulations on Studies on Experimental Animals.

The concentrations of plasma follicle-stimulating hormone (FSH), luteotropic hormone (LH), prolactin, progesterone, and estradiol in the serum were measured by EIA using Stat Fax 2100/2600 system (AWARENESS Technology) and Vector-Best kits. The ovaries, liver, and kidneys for morphological studies were fixed in neutral 10% formalin, the sections were stained with hematoxylin and eosin and by van Gieson method.

The data were mathematically processed directly from Excel 7.0 common data matrix using STATGRAPH 5.1 software. The means, their mean square deviations, and representativeness error were evaluated.

RESULTS

In experimental CET with predominant involvement of the liver, the concentration of medium-molecular-weight substances on day 90 was 0.51±0.06 arb. units, which was 3.18 times higher than in the control group (0.16±0.02 arb. units; p<0.01). In group 2, this value was 0.47±0.09 arb. units, *i.e.* by 2.94 times higher than in intact animals (p<0.01). Similar changes were recorded in both groups for oligopeptide fraction and MDA. Acylase activity in the liver and kidney of experimental animals was significantly reduced compared to intact intact controls.

Morphometry of the liver showed a significant decrease in volume percentage of hepatocytes by day 90 of CET in both groups of experimental animals in comparison with intact rats (Table 1). This shift was paralleled by an increase in volume percentage of the connective tissue in group 1 animals (by 5.18 times; p<0.05) and in group 2 ones (by 4.56 times) and a decrease in the mean diameter of Kupffer cell nuclei.

Similar changes were detected in the kidneys. The volume percentage of the interstitium increased maximally by day 90 of the experiment, surpassing the value in intact rats by 2.74 times in group 1 and by 3.29 times in group 2. These changes were paralleled by shrinkage of the urinary space and reduction of the tubular index (p<0.05) and were more pronounced in group 2.

Changes in the levels of sex hormones over the course of CET indicated disorders in ovarian function (Table 2). The most pronounced changes in serum concentrations of sex hormones were observed mainly for progesterone and prolactin. By day 30 of the experiment, blood progesterone concentration in group 1 and 2 increased by 2.54 and 1.47 times (p<0.05), respectively. High level of progesterone was recorded during all periods of CET in the presence of slight prolactinemia, which attained maximum on day 90

TABLE 1. Morphometric Parameters of CET Severity in Rats (M±m)

Group	Day of study	Morphometric parameters of the liver			Morphometric parameters of the kidneys		
		hepatocyte volume, %	connective tissue volume, %	mean diameter of kupffer cell nuclei, μ	interstitium volume, %	urinary space, arb. units	tubular index, arb. units
Control	–	75.8±9.9	1.6±0.1	23.9±3.6	7.2±0.4	1.4±0.1	0.8±0.1
1	30	79.3±10.4	3.9±0.6	21.5±1.9*	9.2±0.9	1.2±0.1	1.6±0.2
	60	62.9±4.7*	6.2±0.4*	20.9±3.2*	14.5±1.3*	1.2±0.1	0.6±0.1*
	90	56.5±3.9*	8.4±1.2*	18.3±2.9*	19.7±4.4*	1.1±0.6*	0.5±0.1*
2	30	71.1±9.2	3.1±0.2	22.1±1.2	11.1±2.1	1.1±0.2	1.1±0.7
	60	67.3±7.9*	5.5±0.9*	19.8±2.5*	18.1±5.3*	0.9±0.1*	0.5±0.1
	90	59.1±9.7*	7.3±1.1*	19.1±1.7*	23.7±5.3*	0.8±0.1*	0.3±0.1*

Note. Here and in Table 2: *p<0.05 compared to intact rats.

TABLE 2. Changes in Sex Hormone Levels in Rats with CET of Different Severity (*M*±*m*)

Group	Day of study	Progesterone, nmol/liter	Estradiol, pmol/liter	FSH, mU/ml	LH, mU/ml	Prolactin, mU/ml
Control	–	81.4±10.9	71.3±2.0	1.6±0.2	1.0±0.3	45.9±7.0
1	30	206.7±11.3*	68.6±2.9	1.6±0.1	0.9±0.1	44.8±10.7
	60	206.4±10.7*	68.5±3.4	1.5±0.1	0.9±0.1	61.6±5.7*
	90	211.2±6.7*	71.4±3.4	1.5±0.1	0.8±0.1	82.9±4.2*
2	30	119.5±53.7*	68.5±0.1	1.5±0.0	0.9±0.1	43.2±0.0
	60	125.6±21.2*	65.6±6.4	1.5±0.1	0.9±0.1	58.8±5.3
	90	112.2±62.9*	70.1±0.2	1.5±0.1	0.8±0.1	64.9±0.1*

of the experiment in animals of both groups. On the other hand, the concentrations of estradiol, FSH, and LH virtually did not differ from the corresponding parameters in intact rats.

The detected estrogen/gestagen dysproportion was regarded as imbalance of the peripheral sex hormones, indicating functional disorders in the ovaries, developing in CET.

Histological study of the ovarian tissue in group 1 animals on day 30 of the experiment showed signs of hyperemia and well-developed stroma in the ovarian medulla and hilus. The cortical matter contained numerous primordial follicles with characteristic structure (a layer of follicular epithelium, primary oocyte, solitary connective tissue elements). Secondary follicles had mature theca (with inner and outer components). Granular cells were presented by multilamellar cubic epithelium with large hyperchromatic nuclei and slightly basophilic cytoplasm occupying virtually the entire cell volume. Slight vacuolar degeneration of follicular epithelium was seen in some follicles. The follicular cavity was filled with follicular eosinophilic fluid. Tertiary follicles were solitary, with mature theca and several layers of granular cells. The inner granular cell layer was unevenly flattened, the subjacent layers were uneven, with wide cell-cell spaces and appearance of solitary connective tissue cells. The oocyte was located in the center of the follicle and was surrounded by a transparent zone.

On day 60, the ovarian medulla and hilus and the ration of stromal to vascular component in group 1 were similar to those in the control group. The cortical matter contained a negligible number of primordial follicles. Secondary follicles had theca, several layers of granular cells, and proliferating epithelium forming an oviductal tubercle with slight signs of vacuolation. Tertiary follicles with loose thecal layers were peculiar. The outer granular layer was presented by cylindrical epithelium with eccentric large oval nucleus and eosinophilic cytoplasm. Wide cell-cell spaces with separate accumulation of follicular cells were detected between the granular layers. The inner granular layer was presented by a monolamellar cubic epithelium. The majority of atretic follicles had the structure of microfollicular cysts (a cavity with eosinophilic fluid was retained against the background of granular cell membrane lining the follicular lumen) with a thick theca in all atretic follicles.

On day 90, the hilus and medulla of the ovary in group 1 were characterized by slight stromal proliferation. Perivascular reaction with solitary cells of lymphocytic origin was observed in few cases. Changes in the ovarian cortical matter were more pronounced. The counts of primordial, secondary, and tertiary follicles decreased significantly in comparison with day 30, while the count of atretic follicles increased and their structure was partially modified by the follicular cyst type. Ovarian parenchyma was presented by overgrown atretic follicular theca and actively proliferating granular cells with pronounced vacuolar degeneration. Hemorrhages were seen in the cortical tissue (diapedetic and filling the cavities of degenerative follicles).

Histological study of ovarian tissue in group 2 rats on day 30 showed that the hilus and medulla of the ovaries were characterized by slight hyperemia and separate perivascular hemorrhages into the cortical matter. The counts of primordial, secondary, and tertiary follicles with characteristic structure were sufficiently high. Atretic follicles exhibited active proliferation of granular cells, acquiring the lutein type, and thecae cells.

On day 60, pronounced hyperemia of the ovarian hilus and medulla with solitary perivascular groups of lymphocytic cells was seen. The counts of primordial, secondary, and tertiary follicles in the cortical matter was reduced. Secondary and tertiary follicles had well developed theca (with inner and outer compartments), granular cells developed degenerative changes. The oocytes in tertiary follicles were located centrally,

but the nucleoli and transparent membranes were not clearly discernible. Atretic follicles developed significant changes. The majority of them looked like microfollicular cysts surrounded by the theca and were filled with eosinophilic follicular fluid and lined with follicular epithelial cells. Lymphocytic and macrophagic cells were detected in the follicular cystic cavities in some cases. The formation of follicular cysts was paralleled by active growth of parenchymatous elements presented by epithelial and connective tissue (thecal) elements.

On day 90, pronounced hyperemia of stromal vessels was seen in the ovarian hilus and medulla in group 2 rats. Perivascular reaction was slight. More pronounced changes were observed in the ovarian cortical matter. Primordial follicles were solitary. Secondary follicles developed degenerative changes (reduction of the primary oocyte, still located on the oviductal tubercle). The content of follicular fluid was higher than in the control. Granular cells actively proliferated, which was confirmed by their somewhat chaotic location with thickened layer of follicular cells.

Atretic follicles were characterized in the majority of cases by active proliferation of the granular cells and thecal elements with increased lipid content in the lutein cells. In some cases, we observed the formation of follicular cysts presented by the theca with several layers of follicular epithelium under it, with the cavity filled with follicular fluid.

Hence, morphological changes in the ovaries detected in CET were paralleled by hormone imbalance. The presence of a sufficiently representative pool of estrogen-producing cells and phasic changes in the counts of progesterone-producing endocrinocytes corresponded to the hormonal profile and to local aftereffects of hormone imbalance (disorders in the maturing and natural involution of the follicles and activation of substitution stromal proliferation).

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