Morphological and Functional State of Rat Ovaries in the Early and Late Periods after Injection of Vepesid

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Morphological and functional state of the ovaries in female Wistar rats was assessed in the early and late periods after administration of antitumor drug vepesid in a single maximum tolerated dose. In the early period, the drug pronouncedly decreased the number of primordial follicles, bi- and multilayer follicles, and the total number of generative elements. The number of graafian vesicles and corpora lutea did not decrease. In the late period, exhaustion of the reserved potencies of gonads was more pronounced than in the control. Morphological alterations in the ovaries were accompanied by inhibition of the functional state of the female reproductive system in the period corresponding to the action on mature and primordial follicles. This inhibition manifested in increased postimplantation mortality.

Key Words: *vepesid; ovaries; rats*

Sterility is a possible side effect of antitumor drug vepesid on intensively renewing ovarian cells [7,9, 11]. According to clinical observations, assessment of the state of the women reproduction system is carried out after treatment with vepesid as component of complex therapy [7,11] and after monotherapy [9]. Analysis of serum levels of gonadotropic and sex hormones produced controversial conclusions on the degree of ovarian dysfunction after vepesid treatment and on the time of its recovery. Experimental studies showed that single intravenous injection of vepesid to Wistar rats in the maximum tolerated dose did not inhibited hormonal activity of the ovaries [1]. The effect of vepesid on generative status of the animals is little studied.

We examined morphological and functional state of ovaries in Wistar rat in the early and late periods after injection of antitumor drug vepesid.

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MATERIALS AND METHODS

Experiments were carried out on female Wistar rats (n=170) weighing 250 g and aging 2 months. The rats were obtained from Biological Modeling Laboratory of Institute of Pharmacology. Vepesid (etoposide, Teva) was injected to proestrus females in a single maximum tolerated dose (30 mg/kg). The dose was calculated by graphic probit analysis for a 30-day period. The control rats (n=75) received no vepesid. For morphological examination, estrous females were killed by cervical dislocation during 1 month (5 rats per point) and 3 and 6 months postinjection. The gonads were examined on day 1 after cytostatic treatment. To this end, the ovaries were isolated and fixed in Carnoy fluid. Paraffin sections (5 μ) across the entire organ were stained with eosin and hematoxylin. Serial sections were used to calculate structural and functional elements: the number of primordial, bi- and multilayer follicles, graafian vesicles, corpora lutea, and the total number of generative elements [6].

Functional state of rat reproductive system was assessed by copulation ability and efficiency, and by indices of embryonic death. To this end, the females of experimental and control groups were mated with intact males on postinjection days 1-10, 30-40, 90-100, and 180-190 to analyze the gonadotoxic action induced in the mature, primordial, bi- and multilayer follicles. Mating was documented by vaginal smears. The indices of pregnancy and fertility were determined [6]. On pregnancy days 17-20 the females were killed by cervical dislocation. The numbers of corpora lutea in the ovaries, implantation sites in the uterus, and live and dead embryos were calculated per rat. The indices of pre- and postimplantation mortality were determined [6]. The data were analyzed statistically using Mann—Whitney *U* test and Fisher angular transformation.

RESULTS

One day after vepesid injection, interstitial edema and plethoric vessels were observed. The cells of follicular epithelium, theca, and corpora lutea were swollen; the boundaries between these cells were blurred. Karyorrhexis and karyopyknosis were often seen in granulosa cells. In some cases, primordial follicles were presented by one oocyte. Some follicles contained hemosiderin-laden macrophages, which could participate in phagocytosis of surface granulosa cells [8]. At later terms, the revealed alterations were less pronounced. At the same time, the follicular cysts appeared in the ovaries.

The results of quantitative assessment of structural and functional elements of the ovaries are summarized in Table 1. In the early periods after vepesid treatment, the pool of primordial follicles was markedly decreased. One day after the start of the experiment (first estrus), this parameter decreased to 20% of the control value, which could result from cell death in the follicular epithelium and from degeneration of oocytes [3]. Then (experimental day 30, 2-6 estruses) this parameter increased, but did not attain the control level, remaining 2-fold below the control. The increase in this index is related to the formation of new primordial follicles, because their development in rats continues to the age of 3 months [3]. The numbers of bi- and multilayer follicles decreased almost 2-fold on postinjection day 1 (estrus 1) and remained below the normal to the end of the experiment. This is determined by the decrease in the pool of primordial follicles rather than intensification of atretic processes, because the number of atretic follicles did not increase. Most follicles with karyorrhexis of the follicular cells detected on the next day after cytostatic treatment result from the toxic effect of vepesid of granulosa cells, rather than from changes accompanying atresia of the follicles. Vepesid produced no significant changes in the numbers of graafian vesicles and corpora lutea in rat ovaries. The total number of generative elements remained decreased during the entire observation period.

Three and 6 months after the start of the experiment, the ovaries of control rats were characterized by age-related increase in the area of the connective tissue; the number of generative elements decreased. After 6 months, the number of primordial follicles and the total number of generative

TABLE 1. Effect of Vepesid in Maximum Tolerated Dose on the Number of Structural and Functionals Elements of Ovaries ($X \pm m$)

Group and postinjection time		Primordial follicles	Follicles with 2 and more layers of granulosa cells	Atretic follicles	Graafian vesicles	Corpora lutea	Total number of generative elements
Control, days 1-30		1187.50±60.05	173.75±4.26	273.00±7.67	7.50±1.44	10.25±1.65	1652.00±61.51
Experimental	estrus 1	236.25±11.25*	97.50±2.50*	233.00±12.67	6.25±1.25	6.75±0.47	569.75±6.34*
	estrus 2	550.00±38.73*	108.75±10.07*	297.50±45.20	5.00±0.00	6.75±0.85	968.00±76.15*
	estrus 3	572.50±82.80*	122.50±9.46*	283.00±23.66	6.25±1.25	9.50±1.50	994.50±101.07*
	estrus 4	565.00±22.55*	92.00±7.17*	264.00±34.32	6.00±1.00	9.00±1.04	968.25±43.68*
	estrus 6	530.00±55.95*	83.00±7.84*	234.00±20.27	5.00±0.00	6.20±0.48	858.20±53.92*
Control, month 3		806.25±65.49**	156.25±7.46	215.00±12.41	6.25±1.25	7.00±0.91	1190.75±75.01**
Experimental, month 3		517.50±19.84*	91.25±5.15*	236.25±24.35	5.00±0.00	8.75±1.25	866.25±43.25*
Control, month 6		742.50±23.59**	155.00±18.48	261.25±17.72	6.25±1.25	9.00±0.57	1174.00±38.55**
Experimental, month 6		508.00±10.20*	91.25±6.57*	202.50±14.93	6.00±1.00	7.00±0.70	811.00±20.75*

Note. $p \leq 0.05$ compared to *control and **preliminary examination period.

elements decreased by 37% and 26% compared to the corresponding values in 2-month-old rats; the growth of the connective tissue in the ovaries was more pronounced in experimental rats. The number of primordial follicles and the total number of generative elements decreased to 68% and 69% of age-matched control ($p \leq 0.05$). More pronounced exhaustion of ovarian reserve potency observed at the late period in the experimental rats probably results from the toxic effect of vepesid on primordial follicles. We performed a comparative analysis of our results with the data obtained after injection of equivalent doses of antitumor drugs of other groups (platidiam, carboplatin, and farmorubicin) [2,4]. Toxicity of vepesid for ovaries was lower than that of anthracycline antibiotics, and comparable to that of platinum-containing drugs.

The fertility index of experimental female rats did not differ from the control during the entire experiment, which agrees with published data on insignificant inhibition of estrous cycle by vepesid [1]. The efficiency of coupling was similar to that in the control group throughout the experiment. Embryonic death on postinjection months 1 and 3 did not differ from the control. The increase in post-implantation mortality (41.43±6.64 *vs.* 3.41±1.04 in the control, $p \leq 0.05$) was revealed for coupling during experimental days 1-10, which confirms the damaging effect of the drug on mature follicles. This parameter peaked on postinjection day 1, which corresponded to activation of meiotic division in oocytes. The observed phenomenon is characteristic of other groups of antitumor drugs [2,4], and attests to induction of dominant lethal mutations in oocytes of ovulating follicles. High post-implantation death in experimental rats (82.98±5.75 *vs.* 9.75±6.16 in the control, $p \leq 0.05$) was observed, when coupling occurred 6 months after the start of the experiment; this was also documented after administration equivalent doses of antitumor preparations of other groups [2,4,5]. High post-implantation mortality can be caused by disturbances in reparative regeneration in oocytes of primordial follicles. The delayed consequences of the toxic effect of cytostatic preparation in the female organism cannot be excluded.

Thus, degenerative and destructive alterations were revealed in rat ovaries at the early terms after injection of vepesid; some of these changes were reversible. At later terms after injection of the drug, exhaustion of ovarian reserve potency was more severe than in the control. Morphological alterations in the ovaries were accompanied by inhibition of the functional state of the female reproduction system, which manifested in increased embryonic death. This mortality increased during periods corresponding to the toxic action on the primordial and mature follicles.

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