Effect of Paclitaxel on Morphology and Function of Rat Ovaries

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The morphology and function of the ovaries at the early and late terms after single intravenous injection of paclitaxel in the maximum tolerated dose (MTD) were studied in female Wistar rats. Evaluation of the structural and functional elements of the female gonads showed that the targets for the drug were primordial, bi- and multilayer follicles. The number of graafian follicles and corpora lutea did not decrease. A more pronounced (copared to the control) depletion of the ovarian reserve potentialities was detected during the remote period. The drug did not decrease mating capacity and efficiency. On the other hand, high fetal mortality was observed in females treated with paclitaxel, which decreased with lengthening of the period between the cytostatic treatment and mating.

Key Words: *paclitaxel; ovaries; rats*

Introduction of taxanes, created on the basis of crude extract from the bark of Pacific yew (*Taxus brevifolia)*, into clinical practice is a prominent achievement of antitumor chemotherapy [3]. Their high efficiency gave rise to great expectations for the progress in therapy of tumors of many locations, resistant to other cytostatics [1,5,8]. Effective use of drugs of this group in patients of reproductive age after organ-sparing surgery for early forms of ovarian and breast cancer prompted studies of their ovarian toxicity as a side effect on actively regenerating tissues of female gonads [7, 10]. Clinical observations showed that the probability of conception after the treatment became an important problem. It was shown that taxanes damaged rat primordial follicles [11] and suppressed the ovarian endocrine function in experimental animals [2]. Long treatment of female rats with these drugs in low doses led to reduction of fertility [9].

We studied the morphology and function of rat ovaries during the early and late periods after injection of paclitaxel (PTX), an antitumor drug from the taxane group. Since high-dose therapy is used in clinical practice, ovariotoxicity was studied after a single injection of the drug in the maximum tolerated dose (MTD).

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MATERIALS AND METHODS

Experiments were carried out on 175 female Wistar rats (250 g; 2 months). The animals were obtained from Laboratory of Biological Modeling, Institute of Pharmacology. The animals were kept in accordance with the regulations of the European Convention for Protection of Vertebrates Used in Experimental and Other Scientific Purposes (Strasbourg, 1986). Paclitaxel (mitotax, Dr. Reddy) was injected intravenously to female rats during the proestrus phase of the estral cycle in a single dose of 4.6 mg/kg (MTD). The drug dose was calculated by the graphic probit analysis after 30-day observation of animals. Control females (n=75) received no PTX.

For morphological study, ovarian glands were collected during the estrus phase. The animals were sacrificed by cervical dislocation during 1 month

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(5 animals per term) and 3 and 6 months after injection. The ovaries were also examined 24 h after cytostatic injection. The animals were autopsied, the gonads were removed, fixed in Carnoy fluid, 5-μ paraffin sections through the entire organ were stained with hematoxylin and eosin. Structural functional elements of the ovaries were counted in ovarian serial sections: primordial, bi- and multilayer, atretic follicles, graafian follicles, corpora lutea, and total number of generative elements [6].

The endocrine and generative functions of the ovaries were evaluated by the capacity to mating and its efficiency, which were evaluated in terms corresponding to the effects on mature and ovulating follicles (after 1-10 days), bi- and multilayer (days 30-40), and primordial follicles (days 90-100 and 180-190). To this end, experimental and control females (15 per experiment term) were put in cages together with intact males. Mating was determined by vaginal smears. Mating capacity was determined by the ratio of fertilized females to the number of females caged with males (×100), mating efficiency was determined by the ratio of pregnant to fertilized females (×100) [6]. On days 17-20 of pregnancy the females were sacrificed by cervical dislocation and the corpora lutea in the ovary, implantation sites in the uterus, live and dead fetuses per female were counted and the pre- and postimplantation mortality indexes were calculated [6].

The data were processed statistically using Mann—Whitney test and Fisher angular transformation.

RESULTS

Interstitial edema and dilatation of blood vessels were observed in the ovaries early after PTX injection. The follicular epithelial and thecal cells were swollen, the interface between them was blurred. Degradation of the nuclei and death of granular cells and the formation of follicular cysts were seen in many primordial follicles. The inner and outer thecal cells were disorganized. The detected morphological changes were observed during 3 estral cycles after PTX injection. The atretic processes progressed in subsequent days. Macrophage accumulation was detected in some atretic follicles.

The pool of primordial follicles decreased significantly (to 53% of control) during the early period after PTX injection (Table 1). These data are in line with previous reports [1], indicating high sensitivity of primordial follicles to PTX. On the other hand, results of quantitative analysis showed that bi- and multilayer follicles were also the targets for PTX. Twenty-four hours after cytostatic injection their number was 86% of the control, which was presumably due to their partial death. This parameter remained at the level of 60% from the control until day 30 of the experiment, which could result from decreased pool of primordial follicle and intensification of atretic processes. The increase (1.3 times) in the number of atretic follicles in comparison with the control (estrus 3 and 6) is evidence in favor of the latter hypothesis. The number of graafian follicles and corpora lutea in rat ovaries was similar in the compared groups. The total number of generative elements was reduced during all days of the experiment.

Age-specific changes were observed during the late periods of the experiment in control rats: connective tissue growth and reduction of the generative pool. After 6 months the content of primordial follicles was 70%, total number of generative

TABLE 1. Number of Structural Functional Elements of the Ovaries after a Single Injection of PTX in MTD ($X \pm m$)

Time of fixation after PTX injection	Primordial follicles	Follicles with 2 and more layers of granular cells	Atretic follicles	Graafian follicles	Corpora lutea	Total number of generative elements
Control 1 (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
Estrus 1	648.00±11.58*	148.00±7.84*	346.00±30.30	6.00±1.00	9.80±0.58	1157.80±27.44*
Estrus 2	790.00±84.85*	127.00±20.40*	312.00±33.07	6.00±1.00	7.60±0.67	1244.60±133.22*
Estrus 3	654.00±9.80*	126.00±15.44*	361.00±32.91*	6.00±1.00	7.00±1.14	1154.20±44.27*
Estrus 4	635.00±23.27*	110.00±14.28*	288.75±18.75	6.25±1.25	8.75±1.43	1048.75±52.33*
Estrus 6	632.00±5.83*	104.00±7.64*	363.00±52.23*	8.00±1.44	12.80±0.96	1119.80±56.87*
Control 2 (3 months)	844.00±12.08	148.00±14.01	300.00±44.41	6.00±1.00	11.20±0.48	1308.80±59.15
3 months	577.50±23.23*	143.75±9.65	462.50±21.65*	5.00±0.00	9.75±1.11	1198.50±40.52
Control 3 (6 months)	746.67±8.82	126.66±6.00	283.33±10.13	6.66±1.66	10.00±1.52	1173.33±26.58
6 months	520.00±15.28*	86.66±7.26*	266.66±4.40	5.00±0.00	10.75±1.88	890.33±23.38*

Note. *$p<0.05$ compared to the corresponding control.

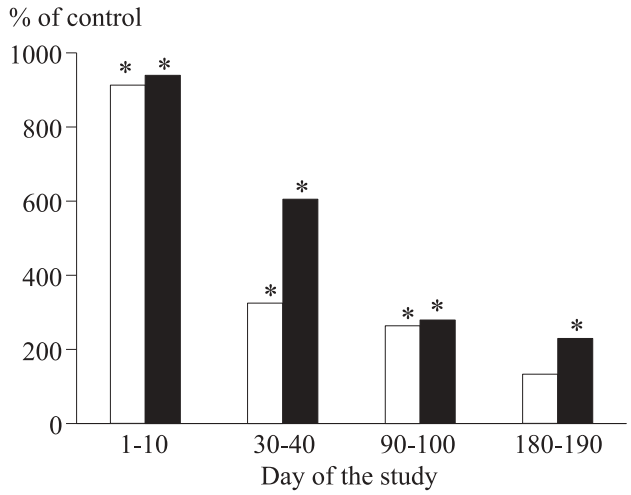

Fig. 1. Pre- (light bars) and postimplantation (dark bars) fetal mortality in female rats injected with PTX and mated during periods corresponding to the effects on different stages of follicle maturation. *$p<0.05$ compared to the control.

elements 76% of that in the ovaries of 2-month-old rats ($p<0.05$).

Three and six months after PTX injection, the content of primordial follicles decreased by 30-32% in comparison with the corresponding control groups (2 and 3). Three months after cytostatic treatment the content of atretic follicles increased (by 50% of control). The numbers of bi- and multilamellar follicles and the total number of generative elements decreased by 30-25% from the control 6 months after PTX injection. At late terms the number of Graafian follicles and corpora lutea was similar to the control values. More pronounced (compared to the control) exhaustion of the reserve potentialities of the ovaries observed during the delayed period after PTX injection was presumably a result of decreased number of primordial follicles observed early after injection of the cytostatic. Comparison of these data with the results of treatment with antitumor drugs of other groups (platidiam, carboplatine, pharmorubicin) in equivalent doses [4] showed lower ovariotoxicity of PTX in comparison with anthracyclin antibiotics, but its toxicity was comparable to that of platinum-containing drugs.

Hormonal activity of the ovaries did not decrease after PTX treatment, because fertility index was similar to the control value throughout the experiment. This is in line with experimental data indicating rapid recovery of the estral cycle after injection of the drug in MTD [2]. Mating efficiency was detected in female rats mated during the early and late periods after injection of PTX.

Embryonic mortality was increased throughout the experiment (Fig. 1). The data attest to decreased probability of pregnancy maintenance. It is noteworthy fetal mortality was high both before and after implantation. Its increase was maximum during the period corresponding to the effects on mature and preovulatory follicles (mating on days 1-10 of the experiment), which was, presumably, due to activation of meiotic division in oocytes of ovulating follicles. High fetal mortality suggests that PTX induces dominant lethal mutations in the maturing follicular oocytes. It is also possible that this toxic effect is due to the destructive effect of PTX on the maternal organism. The increase in fetal mortality was less pronounced with lengthening the period between PTX injection and mating.

Hence, degenerative and destructive changes were detected in rat ovaries during the early period after PTX injection, some of these changes were reversible. The decrease in the number of generative elements led to earlier (compared to the control) exhaustion of reserve potential of the ovaries. No appreciable disorders in their endocrine function after PTX treatment were detected; the probability of pregnancy onset was retained. On the other hand, fetal mortality was high in females injected with PTX. The severity of this toxic effect decreased with lengthening of the period between the treatment and mating.

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