GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Morphological and Functional State of Rat Ovaries in Early and Late Periods after Administration of Platinum Cytostatics

T. G. Borovskaya, V. E. Goldberg, T. I. Fomina, A. V. Pakhomova, S. I. Kseneva, M. E. Poluektova, and E. D. Goldberg

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Experiments on Wistar rats showed that cisplatin and carboplatin induced similar morphological alterations in the ovaries. Both agents reduced the number of structural and functional elements, but the effect of cisplatin was more pronounced. Morphological changes observed in the early period after injection of the preparations were accompanied by prolongation of the estrous cycle, which was longer in rats treated with carboplatin. Partial and reversible sterility was observed in females at the early terms after cisplatin treatment.

Key Words: *cisplatin; carboplatin; ovaries; rats*

Cyclic nature of physiological activity of the ovaries is associated with cyclic changes in their structure and function. Female gonads similarly to other rapidly renewing cell systems are the major targets of the toxic action of antitumor drugs. Recent encouraging results on tumor chemotherapy [5,6] draw considerable attention to the problem of sterility resulting from cytostatic treatment. According to clinical and experimental data, the most pronounced changes in ovarian function were induced by alkylating agents belonging to chloroethane amines, ethylenimines, and ethers of disulfonic acids [3,7,8,11]. Complex platinum compounds belonging to alkylating agents by their basic mechanism of antitumor action are little studied in this respect. It should be emphasized that drugs of this group are used as the basis in various protocols of antitumor chemotherapy [2].

Our aim was to study morphological and functional state of rat ovaries at the early and late periods after administration of first generation platinum preparation cisplatin (cis-diamminedichloroplatinum(II), CDDP) and second generation platinum preparation carboplatin (CP).

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

MATERIALS AND METHODS

The study was carried out on 340 2-month-old female Wistar rats weighing 250 g (Department of Biological Modeling, Institute of Pharmacology). During the proestrus phase of estrous cycle CDDP (Lachema) and CP (ABIK) were injected intravenously in maximum tolerated doses of 4 mg/kg and 60 mg/kg, respectively. The doses were calculated using graphic probit analysis with 30-day follow-up period. The control rats (n=100) received no platinum-based preparations.

For morphological analysis of the ovaries estrous female were sacrificed by cervical dislocation during a one-month period (5 rats per each experimental term) and also 3 and 6 months after administration of the test preparation. The ovaries were fixed in Carnoy fluid, paraffin sections (6 μ) through the entire organ were

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stained with hematoxylin and eosin. In serial sections the following structural and functional elements were counted: primordial follicles (PF), bi- and multilayer follicles, Graafian follicles, corpora lutea, and the total number of generative elements [4].

Functional state of the ovaries was assessed by examination vaginal smears during the estrous cycle. In addition, copulation capacity and efficiency were evaluated. For evaluation of the gonadotoxic effects of the test preparations on mature, bi-, multilayer, and primordial follicles, control and experimental females were cages with intact males on days 1-10, 30-40, 90-100, and 180-190 postinjection. Copulation was verified by vaginal smears. Then fertility and pregnancy indices were determined [4].

The data were processed statistically using Mann—Whitney and Fisher angular conversion tests.

RESULTS

Morphological analysis revealed interstitial edema and dilation of blood vessels in the ovaries 1 month after injection of CDDP and CP. Cells of the follicular epithelium, thecal layers, and corpora lutea were swollen with blurred boundaries. In many PF degradation of cell nuclei and death of follicular epitheliocytes were noted (Fig. 1); follicular cysts appeared (Fig. 2). Degradation of nuclei was also observed in oocytes (Fig. 3). Granulosa of some follicles and the internal thecae had abnormal organization. Pyknosis and degradation were seen in the nuclei of luteal cells, which was accompanied by the formation of cysts. In some atretic follicles we detected macrophage clusters, which could participate in phagocytosis of cells of the surface granulose layer [9]. These changes were observed after administration of both CDDP and CP and were most pronounced on week 1 postinjection (estrus 1 and 2). Three and 6 months postinjection, age-related atrophic processes developed in the ovaries in both groups. However, hyperplasia of the connective tissue in the gonads was more pronounced in experimental rats compared to controls.

During the early period after injection of CDDP and CP, the number of PF decreased by 25-35% (Table 1), which could result from the death of follicular epitheliocytes and degenerative changes in oocytes [1]. The number of bi- and multilayer follicles in the ovaries decreased more than 2-fold in rats injected with CDDP. Damage to PF and multilayer follicles was previously observed after administration of other alkylating agents [3, 11].

In contrast to CDDP, the number of bi- and multilayer follicles increased in the first days after injection of CP (estrus 1 and 2). In addition, the number of atretic follicles decreased in rats treated with CP. The CP-induced stimulation of folliculogenesis evidently caused by hormonal imbalance was reversible.

In later period (estrus 2) we observed drastic acceleration of atretic processes probably caused by hyperstimulation of folliculogenesis. At the early terms after injection of the examined agents, the number of Graafian vesicles decreased insignificantly. The number of corpora lutea was similar in all groups.

These findings suggest that platinum preparations produce less pronounced damaging effect on the ovaries compared to other alkylating substances (*e.g.* cyclophosphamide), which drastically decreased the number of preovulatory follicles and corpora lutea when

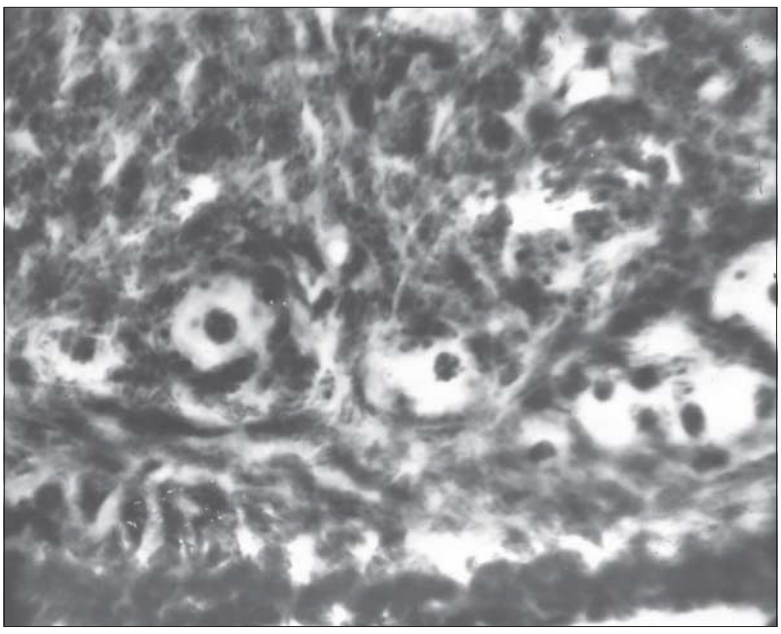

Fig. 1. Effect of carboplatin on rat ovary. Death of follicular epitheliocytes in primordial follicles during estrus 1 postinjection. Here and in Fig. 2 and 3: hematoxylin and eosin staining, ×160.

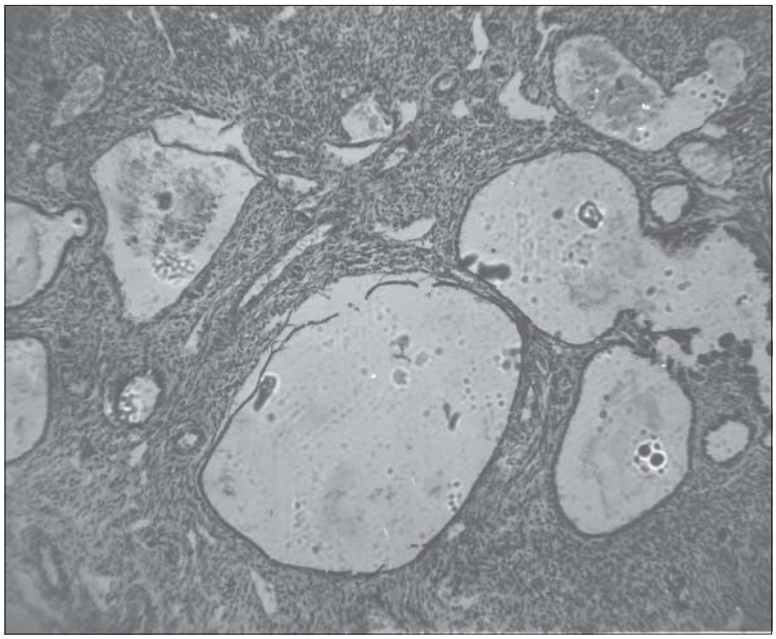

Fig. 2. Effect of cisplatin on rat ovary. Histological picture of follicular cysts during estrus 2 postinjection.

injected in single equivalent doses [8]. The total number of generative elements decreased in both experimental groups on day 1 postinjection. The increase in this parameter in rats treated with CP during the next estrous cycle is probably related to stimulation of folliculogenesis. The increase in the number of generative elements above the control value probably results from the growth of PF pool, because in rats these elements are produced up to the age of 3 months [10].

Both examined cytostatics decreased the number of PF on month 6 postinjection. The number of bi- and multilayer follicles and the total number of generative elements in the ovaries significantly decreased after CDDP treatment. Evidently, earlier exhaustion of adaptive potential in the ovaries at the later terms postinjection resulted from direct damage to PF caused by these cytostatics.

Duration of the estrous cycle in rats treated with PT did not differ from the control value. The second estrous cycle was observed in all rats, but its duration increased due to lengthening of diestrus by 45.14± 20.77 h ($p \leq 0.05$). Then the duration of the estrous cycle returned to normal. In rats receiving CP, hormonal activity of the ovaries was suppressed to a greater degree. In 34% females the estrous cycle was arrested for 24-48 h after injection of CP, while in 64% females

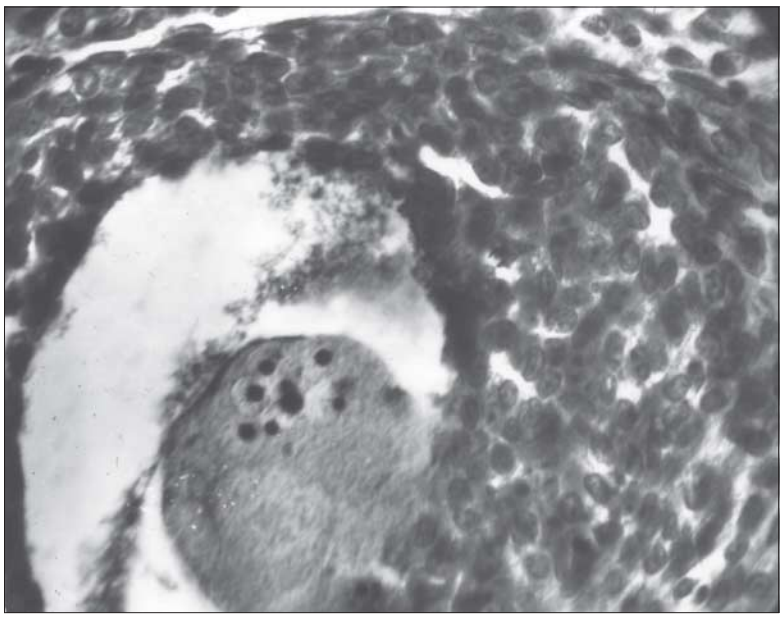

Fig. 3. Effect of carboplatin on rat ovary. Histological picture of karyorhexis in multilayer follicle during estrus 2 postinjection.

TABLE 1. Number of Structural and Functional Elements in Ovaries Before and After Administration of Platinum Preparations ($X \pm m$)

Time postinjection		Index					
		PF	follicles with 2 and more layers of granular cells	atretic follicles	Graafian vesicles	corpora lutea	total number of generative elements
Days 1-30	control	1125.0±131.5	171.3±39.1	286.2±68.8	6.2±2.4	10.3±1.6	1598.0±156.8
	estrus 1	844.0±57.6* 700.0±70.0*	302.0±21.3* 76.7±12.0*	65.0±68.8* 351.7±76.7	4.0±1.9 1.7±1.7	9.2±0.8 7.0±1.1	1225.0±120.2* 1137.0±62.7*
	estrus 2	900.0±71.0 741.7±132.4	450.0±17.8* 125.0±14.4	612.0±75.1* 340.0±61.0	1.3±1.3 2.5±1.4	9.0±0.5 6.2±0.7	1978.0±72.0* 1215.5±160.2
	estrus 3	910.0±90.2 997.0±140.4	250.0±26.0 148.3±6.7	300.0±51.2 318.3±73.6	5.0±2.0 1.7±1.7	11.5±0.9 6.7±0.2	1476.0±160.2 1472.0±183.7
	estrus 4	800.0±20.5* 970.0±140.0	230.0±32.8 140.0±18.6	290.0±47.9 332.5±18.8	6.5±2.2 2.5±2.5	8.2±0.3 8.0±0.4	1336.0±171.4 1452.2±160.8
	estrus 6	700.0±65.0* 625.5±127.8*	230.0±32.2 112.5±17.8	300.0±62.3 415.5±18.8	7.0±2.4 1.2±1.2	9.0±2.3 10.7±1.3	1245.0±114.3 1166.0±73.8
Month 3	control	665.0±99.3	167.5±15.0	542.5±47.9	7.5±2.5	8.0±0.4	1390.0±137.2
	experiment	500.0±90.0 473.3±90.0	171.0±18.5 123.3±22.0	400.0±18.8 350.0±101.0	10.8±3.0 3.3±3.3	9.5±0.8 9.3±0.7	1080.0±130.2 959.3±186.0
Month 6	control	475.0±11.5	160.0±26.0	331.0±65.0	5.0±3.0	6.8±0.3	915.3±165.7
	experiment	350.0±63.0* 267.0±63.0*	200.0±10.5 108.3±10.0*	358.0±73.6 251.7±46.9	6.7±1.7 3.3±1.7	8.5±3.3 8.3±1.8	918.0±145.2 639.7±35.0*

Note. CP and CDDP are shown in nominator and denominator, respectively. *$p<0.05$ compared to the control.

the durations of the estrous cycles 1, 2, 3 increased due to lengthening of diestrus by 15.62±5.88 h. Then phasic structure and duration of the cycle returned to normal. Similar, but more pronounced disturbances in the estrous cycle were observed in rats treated with high doses of Tio-Tef [3]. At the late terms after injection of CDDP or CP the duration of estrous cycle phases was similar to the control values.

Fertility index of experimental females did not significantly decrease throughout the experiment. This can be explained by the fact that inhibition of hormonal activity of the ovaries observed at earlier terms postinjection was transient and the estrous cycle was not disturbed at the later terms.

Partial sterility was observed only after injection of CDDP. The efficiency of copulation during the first 10 day postinjection was only 33% (*vs*. 100% in the control, $p<0.05$). Females fertilized on day 1 after injection of CDDP were nonpregnant, which confirm damaging action of CDDP on preovulatory follicles with activated meiotic fission. At later terms copulation efficiency did not decrease.

Therefore, platinum-based preparations of the first (CDDP) and second (CP) generation induce similar morphological changes and decrease the number of generative elements in rat ovaries at the earlier terms after treatment. At later terms, adaptive reserves of the ovaries are exhausted earlier than in the control. The observed changes were more pronounced in the ovaries of rats treated with CDDP. The morphological aberrations observed at the early terms postinjection were accompanied by prolongation of the estrous cycle, which was more pronounced in rats treated with CP. Partial and reversal sterility was observed at the early terms after injection of CDDP. Comparison of the present and published [3,8] data shows that complex platinum compounds produced less pronounced damaging effects on female gonads compared to other alkylating agents.

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