MORPHOLOGY AND PATHOMORPHOLOGY

Morphofunctional Status of the Ovaries in Platidiam-Treated Rats

E. D. Gol'dberg, T. G. Borovskaya, T. I. Fomina, and M. E. Smirnova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 11, pp. 571-573, November, 1996
Original article submitted April 30, 1996

Intravenous injection of the maximum tolerated dose of platidiam prolongs diestrus in rats. Degenerative changes develop in the ovaries containing fewer generative elements. These changes are reversible.

Key Words: *rat; estrous cycle; ovary; platidiam*

Recently, the problem of sterility as a consequence of chemotherapy has attracted considerable attention [8]. It was reported that cytostatics cause oligomenorrhea and premature menopause [8,9]. Abnormalities of the estrous cycle, intensification of atretic processes, and death of primordial and multilayer follicles have been described in rats given antimetabolites, alkylating compounds, or anthracycline antibiotics [3,4,7]. In this study we examined the effect of platidiam, a platinum-containing cytostatic widely used in oncologic practice, on the morphofunctional status of the ovaries of Wistar rats.

MATERIALS AND METHODS

The study was conducted on 80 Wistar rats (body weight around 200 g) with an established estrus cycle; 15 of these rats served as controls. Platidiam (Lachema) was injected once intravenously in the maximum tolerated dose (MTD) of 4.5 mg/kg at the proestrus stage. This dose was calculated by graphical probit analysis [1] after observing the animals for 30 days. The ovarian function was assessed by examination of vaginal smears [6].

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

Rats were killed by cervical dislocation over a 30-day period after platidiam injection to analyze morphological abnormalities arising in the ovaries during estrus. The ovaries were removed and fixed in Carnoy's fluid. Paraffin sections (6 μ) were cut throughout the ovary and stained with hematoxylin and eosin. Primordial follicles, follicles with one, two, or more layers of granulosa cells, mature follicles (graafian vesicles), atretic follicles, corpora lutea, and generative elements were counted. Counts were made over the entire surface of the section: primordial follicles and those with one layer of granulosa cells were counted in every 10th section and the result was multiplied by 10; mature follicles and those with two or more layers of granulosa cells were counted in every 5th section, while corpora lutea were counted in the middle section. Follicles with the oocyte containing nucleus and nucleolus were taken into account [6]. The significance of differences was evaluated by the Wilcoxon—Mann—Whitney test.

RESULTS

In control rats, the mean duration of the estrous cycle was 104.33±1.87 h (diestrus lasted 57.83±1.87 h, proestrus 15.00±1.33 h, estrus 26.00±1.00 h, and metestrus 6.50±0.34 h). In platidiam-treated rats,

0007-4888/96/0011-1144$15.00

the duration of the first estrous cycle after the drug administration was similar to that in control rats. The second estrous cycle occurred in all females, but in 30% of them estrus was shorter by 11.67±4.98 h than in the controls. The duration of diestrus increased in all platidiam-treated rats: by 18.50±2.02 h in 70% and by 121.00±29.60 h in 30% of the animals. In the third estrous cycle, the diestrus was longer in 40% of the rats (by 28.67±6.86 h). Subsequently, the estrous cycle in the platidiam-treated females was similar to that in the controls.

Morphological analysis revealed hemodynamic disorders (vasodilation, edema, and lymphoid cell infiltration) which developed in the ovaries during a 30-day period after platidiam injection. Cells of the follicular epithelium, corpora lutea, and thecae were swollen. These changes were most pronounced during the first week after platidiam administration (the first and second estrus). Platidiam produced the highest toxic effect on primordial follicles: their number dropped >1.5-fold as early as 24 h (first estrus) after the drug administration (Table 1). Destruction of the primordial follicle started from the oocyte death: its nucleus became shrunken and pyknotic. Follicular cells retained their integrity for some time, but then disappeared. During the second, third, and fourth estrus, the amount of follicles in platidiam-treated rats remained below that in the controls, the difference being statistically insignificant. One month after platidiam injection (the sixth estrus), the number of primordial follicles was still significantly lower than in the control rats (Table 1). Multilayer follicles were also sensitive to platidiam. As early as 24 h after platidiam injection, their number decreased more than 2-fold compared with the controls. Pyknosis, nuclear breakdown, and massive death of follicular epithelial cells were observed at that and later time periods in many antral and late preantral follicles (Fig. 1). In some follicles,

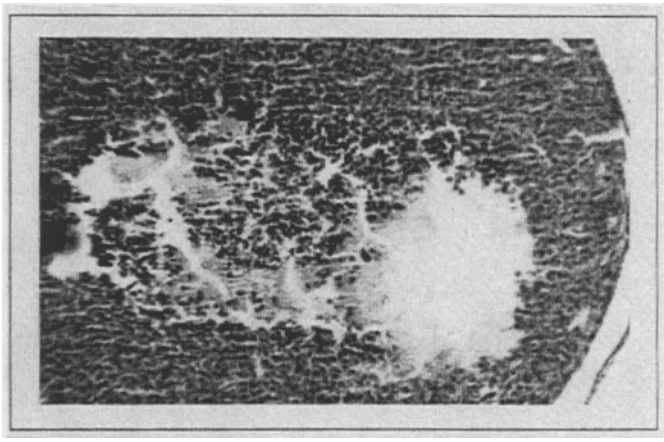

Fig. 1. Massive death of follicular epithelial cells in rat ovarian follicle during the first estrus after administration of platidiam in the MTD. Here and in Figs. 2 and 3: hematoxylin and eosin staining, ×160.

the granulosa and internal theca were disrupted. In multilayer follicles, the oocytes were degraded and looked like a dark lump. A late preantral follicle with two oocytes was found 24 h after platidiam injection. Follicles with multiple oocytes arise because the adjacent oocytes are not completely surrounded by follicular cells [2]. Throughout the observation period, the ovaries of platidiam-treated rats contained fewer mature and atretic follicles and corpora lutea compared with the control (Table 1). However, the differences were insignificant due to a wide variation range. This may be associated with individual sensitivity to platidiam, since the length of estrous cycle varied from animal to animal.

In control rats, the granulosa of atretic antral and late preantral follicles was retained and the theca was hypertrophic. In the ovaries of cytostatic-treated rats, atresia was more pronounced, manifesting itself as degeneration of the granulosa and marked hypertrophy of the theca (Fig. 2). During the first week after platidiam administration (first and second estrous cycles), pronounced pyknosis

TABLE 1. Numbers of Structural/Functional Elements in the Ovaries of Rats After a Single Administration of Platidiam in the MTD (*M±m*)

Elements	Control group	Time after administration				
		1st estrus	2nd estrus	3rd estrus	4th estrus	6th estrus
Primordial follicles	1125.0±131.5	700.0±70.0*	741.7±132.4	997.0±248.9	970.0±140.4	627.5±127.8*
Follicles with 2 or more layers of granulosa cells	171.2±39.1	76.7±12.0*	125.0±14.4	148.3±6.7	140.0±18.6	112.5±17.8
Graafian vesicles	6.2±2.4	1.7±1.7	2.5±1.4	1.7±1.7	2.5±2.5	1.2±1.2
Corpora lutea	10.25±1.6	7.0±1.1	6.2±0.7	6.7±0.2	8.0±0.4	10.0±1.3
Atretic follicles	286.2±68.6	351.7±76.7	340.0±61.0	318.3±73.6	332.5±18.8	415.0±100.0
Total number of generative elements	1599.0±156.8	1137.0±62.7*	1215.5±160.2	1472.0±183.7	1452.2±160.8	1166.0±73.8

Note. *$p \leq 0.05$ compared with the control group.

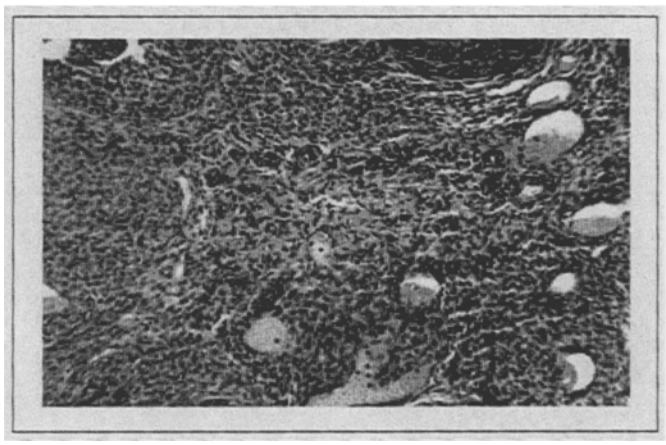

Fig. 2. Massive follicular atresia in a rat ovary during the first estrus after administration of platidiam in the MTD. Pronounced hypertrophy of the theca.

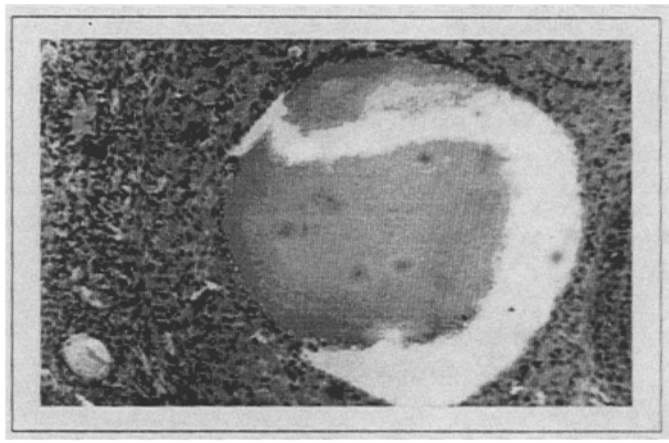

Fig. 3. Cyst of the corpus luteum in a rat ovary during the second estrus after administration of platidiam in the MTD.

and breakdown of the nuclei as well as emergence of necrosis areas were observed. Cysts developed in some corpora lutea (Fig 3). Twenty-four hours after platidiam injection (first cycle), the total number of generative elements was significantly lower compared with the control (Table 1), apparently due to a decrease in the number of primordial, two-layer, and multilayer follicles. Subsequently, this parameter remained lower than in the control.

Thus, a single injection of the MTD of platidiam to female rats prolonged the estrous cycle as a result of increased duration of diestrus. Morphological analysis showed that this cytostatic causes reversible degenerative changes in the ovaries. Primordial, two-layer, and multilayer follicles proved to be highly susceptible to platidiam. Since the level of estrogen secretion is highest at the end of diestrus and during proestrus [5], i.e., during the period of intense growth of the follicle, a decrease in the number of follicles may be one of the reasons for prolongation of the estrous cycle.

REFERENCES

1. M. M. Belen'kii, in: *Pharmacological Effects: Elements of Quantitative Estimation* [in Russian], Leningrad (1963), pp. 81-106.
2. L. F. Kurilo, *Arkh. Anat.*, No. 8, 63-69 (1980).
3. E. P. Lander, *Zh. Obshch. Biol.*, No. 1, 121-125 (1968).
4. A. G. Savitskii, R. D. Ivanova, and A. I. Nikitina, *Vopr. Onkol.*, **25**, No. 3, 84-86 (1977).
5. V. M. Samsonov, *Probl. Endokrinol.*, **23**, No. 2, 113-122 (1977).
6. I. V. Sanotskii and V. N. Fomenko, in: *Long-Term Effects of Chemical Compounds on Living Systems* [in Russian], Moscow (1970), p. 84.
7. M. V. Fillipova, T. I. Fomina, and E. A. Andreeva, in: *Topics of Current Interest in Pharmacology, with Special Emphasis on Issues Relating to the Quest for New Drugs* [in Russian], Vol. 5, Tomsk (1992), pp. 23-25.
8. D. Marmor, *Contracept. Fertil. Sex*, **21**, No. 10, 739-743 (1993).
9. J. L. Miller, *Cancer Bull.*, **44**, No. 2, 154-155 (1992).