Pathomorphological Study of the Cytotoxic Effects of Cyclic Polychemotherapy on Reproductive Organs of Female Rats

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The effects of cyclic polychemotherapy on reproductive organs were studied in female rats intraperitoneally injected (twice at 7-day intervals) with cyclophosphamide (21 mg/kg), adriamycin (2.1 mg/kg), vincristine (0.04 mg/kg), and prednisolone (2.1 mg/kg). Changes in the vaginal swabs on day 1 after polychemotherapy corresponded to estrus in 45% animals, to proestrus in 25%, and were undifferentiated in 30%. After repeated injection of the cytostatics, undifferentiated changes were found in 80% females and persisted for 6 days. Injection of the cytostatic complex led to degenerative changes and necrosis of follicular epithelial cells, edema and focal necroses of vascular endotheliocytes, which progressed after repeated polychemotherapy. By the end of 1.5 months following 2 courses of polychemotherapy, the numerical density of primary, early and late secondary follicles in the ovaries decreased and vascular sclerosis developed indicating progressive atrophy.

Key Words: *cytostatics; polychemotherapy; ovaries*

An overall increase in the incidence of malignant tumors is observed in recent years. Introduction of new antitumor agents and polychemotherapy (PCT) cycles in practical oncology improves the treatment of malignant tumors, leading to better results and appreciable prolongation of patient's lifespan. Modern antitumor therapy is characterized by high aggressiveness and toxicity influencing not only tumor, but also normal tissues [1,3], and causing structural and functional disorders in the reproductive organs [2,4,6].

This problem is particularly significant for young people, for whom the severity of developing postcytostatic complications in the reproductive organs determines future quality of life. Almost 50% of all patients with Hodgkin's lymphoma are females, 81% of them are aged 15-36 years, and hence, preservation of their reproductive function is an important problem [4]. The absence of sufficient information on the type of lesions in the rapidly proliferating organs of the reproductive system and regeneratory processes in them during the postcytostatic period after repeated PCT courses necessitates thorough experimental study of these aspects.

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MATERIALS AND METHODS

The experiment was carried out on 50 female adult Wistar rats (180-200 g). The animals were handled in accordance with the regulations adopted by the European Committee for Protection of Vertebrates Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The animals received two injections (at 7-day interval) of the complex of drugs used for the treatment of hemoblastosis by the CHOP protocol: cyclophosphamide (21 mg/kg), adriamycin (2.1 mg/kg), vincristine (0.04 mg/kg), and prednisolone (2.1 mg/kg). The chosen dose was $^1/_5$ LD_{50}.

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Cytological analysis was carried out on the material collected by vaginal swabbing, carried out daily from day 1 and during 6 days after repeated injection of the cytostatics until the end of the experiment. Cytological smears were stained with azur-eosin by the method of Leishman; the estrus and proestrus phases were verified by changes in hormone concentrations.

The animals were decapitated (under ether narcosis) 1, 7, and 14 days, 1 and 1.5 months after repeated PCT. Both ovaries were collected for histological study, fixed in 10% formalin, histological preparations were made by the standard method, and paraffin sections (5-6 μ) were stained with hematoxylin and eosin and by van Gieson method [5].

Structural organization of the ovarian tissue was examined under a light microscope at magnification up to ×1000; numerical density (ND) and proportion of primary follicles, early and late secondary follicles, and follicular cysts (medium and large antral follicles undergoing cystic atretic transformation) were evaluated at ×400, and the follicle diameters were measured.

The data were processed statistically using Student's *t* test; differences between the means were considered significant at $p<0.05$.

RESULTS

The vaginal epithelium is a good indicator of cyclic hormonal changes: by the moment of the cytostatic complex injection, 60% females were in the estrus phase and in 40% the changes corresponded to the proestrus phase (Fig. 1). One day after the first injection of the complex, the changes in 43% females were identified as the estrus phase, in 25% as proestrus, and in 30% were undifferentiated, because the cytogram of the vaginal epithelial cells showed no cytological signs characteristic of a certain hormonal phase; squamous epithelium apoptosis (pyknosis, fragmentation, margination of the nucleus and apoptotic bodies) and a significant increase in the number of neutrophilic leukocytes in the cytological preparations were observed.

After repeated injection of the cytostatics, undifferentiated changes were observed in 80% females, and during subsequent 3 days minimum changes characteristic of estrus or proestrus were seen in 50% animals. However, by day 5, when a new estral cycle was to begin, 85% females again exhibited undifferentiated changes and only 15% had proestrus signs, but no estral activity (Fig. 1). On day 6 after repeated PCT, no estral activity was detected, but the number of females with changes characteristic of proestrus increased 2-fold.

Hence, by the end of the week after repeated injection of cytostatics, the cytological signs of estral effects on the vaginal epithelium disappeared almost completely; only cells with progesterone transformation and undifferentiated changes.

Double injection of a complex of cytostatics led to reduction in all types of follicles: after 1 day, ND of follicles was 83.6% of control, after 7 days 74.4%, after 14 days 87.3%, after 1 month 79.3%, and after 1.5 months 53.7% of control (Table 1).

ND of primary follicles maximally dropped during the early period after 2 courses of PCT: by 1.2 times compared to the control on day 1 and 1.3 times on day 7, while ND of secondary follicles remained at the same level as in the control (Table 1). This proves the direct cytotoxic effect of PCT on cells with high proliferative activity, including cells in

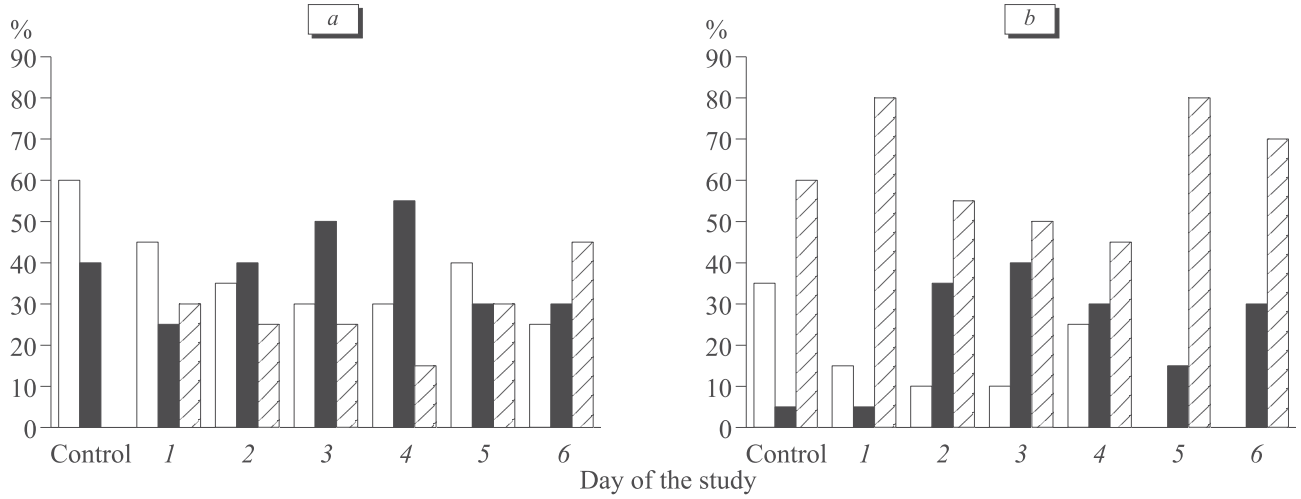

Fig. 1. Cytograms of vaginal smears of female rats. *a*) PCT course 1; *b*) PCT course 2. Light bars: estrus; dark bars: proestrus; cross-hatched bars: undifferentiated changes.

TABLE 1. ND and Diameters of Ovarian Follicles (*M*±*m*)

Day of experiment	Follicles ND					Diameter of follicles, μ
	total	primary	secondary		follicular cysts	
			early	late		
Control	86.20±16.44	16.30±0.52	22.20±5.44	36.10±0.53	14.10±9.35	18.20±0.98
Day 1	72.10±17.37	11.90±5.65*	25.10±7.47	23.40±6.88	12.30±9.13*	17.70±0.95
Day 7	64.20±23.46	10.20±9.15*	16.50±4.07	18.10±7.75*	20.20±9.73	15.30±1.15
Day 14	75.30±25.17	24.50±8.23	15.50±7.83*	20.40±9.22	16.50±10.48	10.20±1.19*
1 month	68.40±16.62	21.20±10.44	24.60±7.17	12.90±5.63*	9.60±5.31*	9.60±0.97*
1.5 months	46.30±20.53*	15.40±11.16	15.30±11.10	12.20±5.42*	13.2±12.4	11.50±1.03*

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

the ovaries. After 1.5 months despite the fact that the total number of follicles constituted $^1/_2$ of their count in the control, the maximum increment in the parameter was at the expense of the primary follicles, their ND reaching virtually the control level, while ND of late secondary follicle was minimum (the regeneration processes just started by this period). Low ND values for all types of follicles 1.5 months after two PCT courses and predominating follicular cysts indicated the absence of full-value reparative regeneration during this period (Table 1).

The diameters of follicles lined by the follicular epithelium (FE) also progressively decreased during the postcytostatic period, which was due to FE necrosis. One day after the injection, this parameter decreased by 2.7% in comparison with the control, after 7 days by 16%, after 14 days by 44%, and after 1 month by 47%. After 1.5 month, a trend to recovery of FE cells was noted: the diameter of follicles reached 11.50±1.03, which by 20% surpassed the minimum diameter observed during the previous period (1 month after treatment; Table 1). Reduction of follicular ND in the ovaries correlated with changes in the follicular diameter.

As soon as the follicle enters the phase of development, it is "destined" to ovulation within a certain period, and this sequence of events cannot be arrested or delayed. Follicular epithelium is characterized by high proliferative potential, and is therefore vulnerable to nonselective effects of antiblastoma agents even after a single injection of cytostatics [3]. Two PCT courses in our study significantly reduced the number of primary follicles in the ovary, because the cytostatics caused necrosis of the follicles which had started the developmental phase or of those which were in the initial phase or stimulation, and these cells died (became atretic), instead of continuing further differentiation.

The detected changes confirm the direct cytotoxic effect of PCT on FE, progressing with every next course. Insufficient estrogenic saturation during PCT and appearance of undifferentiated changes in the vaginal epithelium indicated impairment of the hormonal function of the gonads by the hypoestrogenic single-phase cycle type [7,8].

One and a half months after PCT, atrophic processes in the ovaries presented by reduction of ND of the primary, early and late secondary follicles, and vascular wall sclerosis. However, signs of compensatory adaptive processes appeared at this time, presenting by angiomatosis of the ovarian microcirculatory network and increase in ND of the primordial follicle, this suggesting possible later improvement of the ovarian structure and function.

Hence, repeated injection of antitumor drugs belonging to different classes of chemical compounds caused lasting disorders in the morphology and function of the reproductive organs of female rats.

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