PHARMACOLOGY AND TOXICOLOGY

The Stage of Offspring of Female Rats after Treatment with Cytostatics of Various Groups

T. G. Borovskaya, A. V. Perova, A. V. Pachomova, M. E. Poluektova, and V. E. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 10, pp. 409-412, October, 2008
Original article submitted April 2, 2008

We examined the offspring of female rats that were mated with intact males in the delayed period after administration of cytostatic drugs Farmorubicin, platidiam, carboplatin, etoposide, and paclitaxel (1, 3, and 6 months post-treatment). Toxicity of these drugs in the offspring decreased in the following order: paclitaxel>etoposide>carboplatin>platidiam>Farmorubicin. The toxic effect depended not only on the type of cytostatic treatment, but also on the period of conception.

Key Words: *cytostatic drugs; female rats; delayed consequences; offspring*

Cytostatic chemotherapy is usually followed by reversible reproductive dysfunction in young women [6,7]. During long-term remission, the majority of female patients desire to have children. Taking into account the diagnosis and therapy, several disorders not associated with the underlying disease are of considerable importance. The risk of defective offspring is high. Cytostatic drugs are genotoxic compounds that can cause cytogenetic disorders in maturing oocytes [2]. These changes can be followed by death of the fertilized ovum and spontaneous abortion [8]. However, good evidence exists that childbirth can occur in women with a history of cytostatic treatment. There are conflicting data regarding the offspring of these patients [7,8], which probably results from differences in chemotherapeutic regimens.

Here we examined the offspring of female rats that were treated with antitumor drugs of various chemical classes.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk, Russia. ***Address for correspondence:*** mmu@pharm.tsu.ru. T. G. Borovskaya

MATERIALS AND METHODS

Experiments were performed on 300 adult female Wistar rats aging 2 months, weighing 250 g, and obtained from the Laboratory of Biological Modeling (Institute of Pharmacology, Tomsk Research Center). Our study was conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The desire to conceive among women usually arises in the delayed period after chemotherapy (3, 5, and 10 years). In our experiments, the animals were mated 1, 3, and 6 months after cytostatic treatment. Further examination was performed with 3200 fetuses and 650 rat pups.

Experiments were conducted with the following cytostatic drugs: anthracycline antibiotics, Farmorubicin (FR, Farmitalia Carlo Erba); complex platinum compounds, platidiam (ABIK Ltd.) and carboplatin (CP, Dabur India Ltd.); topoisomerase activity inhibitors, etoposide (Teva); and taxanes, paclitaxel (PT, Dr. Reddy's). They are widely used in clinical practice. Female rats received single

intravenous injection of drugs at the maximum tolerable dose (high-dose therapy in clinical practice). An equivalent volume of the solvent was administered to control rats (n=150).

Female rats of the treatment and control groups were mated with intact males 1, 3, and 6 months after the start of the study (20 specimens for each time point). The mating was detected by examination of vaginal smears. The fetuses were studied macroscopically. The internal organs of viable offspring were examined during the antenatal period (day 20 of pregnancy) by the method of Wilson. Ossification was studied by the method of Dawson [3]. The survival rate, physical development, appearance of sensory and locomotor reflexes (precipice avoidance test, turning over on the plane, muscle strength, and open-field test), learning capacity (conditioned passive avoidance task) [5], and adaptive behavior (stress avoidance by the method of Henderson with modification of N.A. Bondarenko) [1]) were studied in the postnatal period. The severity of pathological changes in the offspring was expressed in arb. units.

The results were analyzed using Mann—Whitney test and Fischer's angular transformation.

RESULTS

Viable offspring was obtained after mating of female rats from all groups. Survival rate was decreased in the offspring of female rats receiving CP, PT, and etoposide (Table 1).

External abnormalities were revealed after treatment with platidiam and etoposide (specimen with 1 head and 2 trunks; and specimen with hypoplasia of the testicles). The number of fetuses with pathological changes in internal organs was high in all experimental groups (except for the offspring of platidiam-treated rats). The majority of specimens had hemorrhages in organs and tissues, cholestasis, hydrocephaly, and nephroptosis. Inhibition of ossification, delayed development of sensory and locomotor reflexes, and reduced capacity for adaptive behavior were found after cytostatic treatment (Table 1). Hence, these drugs differ in the latency of toxicity and toxic influence on the offspring.

It was interesting to evaluate individual characteristics of the offspring from various treatment groups. The offspring of CP-treated females had the lowest survival rate. The offspring of etoposide-treated females was characterized by the highest incidence of pathological changes in internal organs, delayed development of sensory and locomotor reflexes, and decrease in muscle tone. High survival rate was typical of the offspring from females that received FR and platidiam. Physical retardation, disturbances in the open-field behavior, and reduction of learning capacity were not revealed in the offspring of FR-treated females. A wide range of pathological changes was observed in the offspring of PT-treated females during all periods of study.

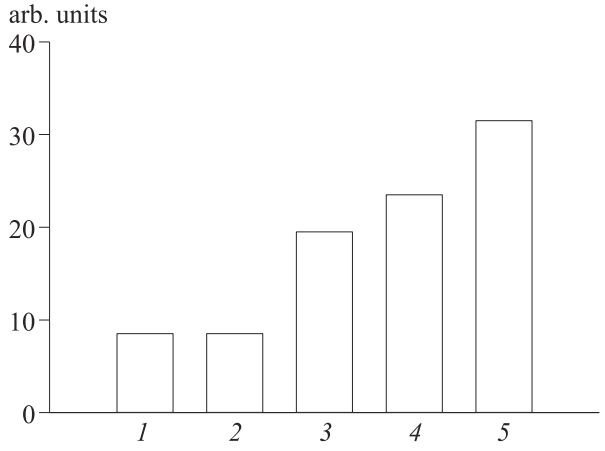

Fig. 1. Integral quantitative evaluation of the offspring from female rats after treatment with antitumor drugs of various groups (all periods of examination). Here and in Fig. 2. FR (*1*), platidiam (*2*), CP (*3*), etoposide (*4*), and PT (*5*).

Comparative integral study of viable offspring (all periods of mating) showed that toxicity of the test drugs decreases in the following order: PT>etoposide>CP>platidiam>FR (Fig. 1). The overall severity of pathological changes in the offspring was highest after treatment with the most toxic drugs.

An integral study was performed taking into account the period of mating. It was shown that the severity of toxic changes in the offspring depended not only on the type of cytostatic treatment, but

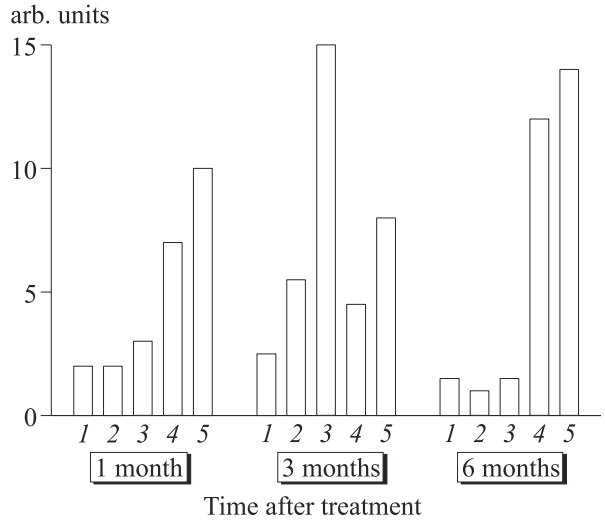

Fig. 2. Integral quantitative evaluation of the offspring from female rats: drug treatment 1, 3, and 6 months before mating.

TABLE 1. Pathological Changes in Viable Offspring of Female Rats after Mating in the Post-Treatment Period

Parameter	Time after treatment														
	1 month					3 months					6 months				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
External abnormalities	—	+ (1)	—	+ (1)	—	—	—	—	—	—	—	—	—	—	—
Pathological changes in internal organs	+ (1)	—	++ (2)	++ (2AB	+ (1)	+ (1)	—	+ (1)	++ (2)	+ (1)	—	—	+ (1)	++ (2)	+ (1)
Ossification	+ (0.5)	—	+ (0.5)	+ (0.5)	++ (1)	+ (0.5)	++ (1)	—	+ (0.5)	++ (1)	+ (0.5)	+ (0.5)	—	++ (1)	+ (0.5)
Dynamics of body weight	—	—	—	—	+ (1)	—	+ (1)	+ (1)	—	+ (1)	+ (1)	—	—	—	+ (1)
Survival rate	—	—	—	—	+ (3)	—	—	++++ (12)	—	—	—	—	—	+ (3)	++ (6)
Physical development	—	—	—	—	+ (1)	—	+ (1)	—	—	+ (1)	—	—	—	+ (1)	+ (1)
Muscle tone	—	+ (1)	—	+ (1)	+ (1)	—	+ (1)	—	—	+ (1)	—	—	—	++ (2)	+ (1)
Reflexes of turning over on the plane and precipice avoidance	—	—	—	+ (1)	+ (1)	+ (1)	—	+ (1)	—	+ (1)	+ (1)	—	—	++ (2)	+ (1)
Open-field behavior	—	—	—	+ (0.5)	—	—	+ (0.5)	*	++ (1)	++ (1)	—	—	—	+ (0.5)	+ (0.5)
Learning capacity	—	—	+ (0.5)	+ (0.5)	+ (0.5)	—	—	*	+ (0.5)	+ (0.5)	—	—	—	+ (0.5)	+ (0.5)
Adaptive behavior	++ (1)	—	—	+ (0.5)	+ (0.5)	—	++ (1)	*	+ (0.5)	+ (0.5)	—	+ (0.5)	+ (0.5)	+ (0.5)	++ (1)

Note. "-", no toxic effect (by test parameter); "+" and "++++", toxic effect of different degree. FR (*1*), platidiam (*2*), CP (*3*), etoposide (*4*), and PT (*5*). *No offspring. Toxic effect (arb. units) is shown in brackets.

also on the period of conception (Fig. 2). The period of mild toxic symptoms was different for various drugs. The optimal time of mating for females of the etoposide and PT groups was 3 months post-treatment. This period for female rats receiving platinoids and anthracycline antibiotics was 6 months. Taking into the account the length of the reproductive cycle in rats [4], these periods correspond to 5 and 10 years of life in humans. These data can be useful to reduce the risk of defective offspring in clinical practice.

Pathological changes in the offspring are probably related to the mutagenic effect of antiblastic drugs on maturing oocytes. Our hypothesis is confirmed by the fact that the offspring of male rats receiving these drugs in equivalent doses have similar disturbances. However, the severity of disorders was much higher after cytostatic treatment of female rats. These features are probably associated with higher sensitivity of female gametes to the adverse effect of drugs. Moreover, cytostatic drugs have a toxic effect on the maternal organism.

Our results indicate that the offspring of female rats receiving cytostatic drugs at the stage of progenesis is characterized by higher incidence of pathological changes. The severity of disorders depends on the chemical structure of drugs and period of mating.

REFERENCES

1. E. D. Gol'dberg and T. G. Borovskaya, *Gonadotoxic Effects of Antitumor Drugs* [in Russian], Tomsk (2000).
2. A. D. Durnev and S. B. Seredenin, *Mutagens. Screening and Pharmacological Prevention of the Effects* [in Russian], Moscow (1998).
3. S. M. Langa and R. P. Wilson, *Praktikum. Laboratornye Zhivotnye*, **2**, 111-120 (1993).
4. *Manual on Experimental (Preclinical) Study of New Pharmacological Substances*, Ed. R. U. Khabriev [in Russian], Moscow (2005).
5. D. E. Shilin and E. V. Ignashina, *Probl. Endokrinol.*, **45**, No. 6, 36-42 (1999).
6. A. V. Hoekstra, P. Keh, and J. R. Lurain, *J. Reprod. Med.*, **49**, No. 6, 447-452 (2004).
7. K. Kanazawa, T. Suzuki, and K. Sakumoto, *Am. J. Clin. Oncol.*, **23**, No. 3, 244-248 (2000).
8. D. Marmor, *Contracept. Fertil. Sex*, **21**, No. 10, 739-743 (1993).
9. J. M. Schilder, A. M. Thompson, P. D. DePriest, *et al.*, *Gynecol. Oncol.*, **87**, No. 1, 1-7 (2002).