Effects of Antitumor Drugs on Offspring

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The offspring of Wistar rats obtained from parents, one of which received platinum-containing drug Platidiam (cisplatin) or anthracycline antibiotics Doxorubicin and Farmorubicin was characterized by low viability (embryonic death increased 4-30-fold compared to control) and congenital malformations. The fetuses displayed pathology in internal organs and low ossification rates. Newborn rats are characterized by low survival rate and retarded physical development.

Key Words: *Platidiam; Doxorubicin; Farmorubicin; offspring*

The literature described many cases of childbirth in patients in complete and long-term remission after cytostatic therapy for trophoblastic tumor, testicular and prostatic cancer, lymphogranulomatosis, acute lymphoblast leukemia, and breast cancer [4-6,9,10]. These malignancies are most sensitive to cytostatic drugs, and chemotherapy not only prolongs patients lifespan but, sometimes, cures the patients [3]. However, antitumor agents cause cytogenetic disorders in normal (not transformed) cells, including germ cells [11]. There is contradictory information on the offspring of parents who received anticancer drug therapy. For example, S. Retsas *et al.* [8] reported that the incidence of developmental disorders in these children is within the normal range; however, there are reports on stillbirth, malformations, and high incidence of malignant tumors in offspring [7].

Here we examine the offspring of rats injected 1, 3, and 6 months before mating with anticancer drugs of different groups: the anthracycline antibiotics Doxorubicin and Farmorubicin or platinum derivative Platidiam (cisplatin).

MATERIALS AND METHODS

Experiments were performed on 120 male and 480 female Wistar rats aged 2 months and weighing 200-250 g (Rassvet nursery, Tomsk). The control group consisted of 300 rats (60 males and 240 females). Before the experiment the rats were allowed 2 weeks of acclimatization. Before and during the experiment, the rats were kept in a vivarium in standard plastic cages (no more than 15 animals per cage) with fine wood chips at 20-22°C, 50% humidity, 8:10 exhaust/inflow ventilation, and natural light/dark regimen. The rats received standard granulated PK120-3 chew. A total of 2800 fetuses and 2880 rat pups were examined to determine the state of the offspring. The rats received single intravenous injections of anticancer drugs cisplatin (Platidiam, Lachema), Farmorubicin (Farmitalia Carlo Erba), and Doxorubicin (Adriablastin, Farmitalia Carlo Erba) in a maximum tolerated doses determined by graphical probit analysis after 30 days of follow-up [1]. Drug-treated males and females were mated to untreated (intact) partners 1, 3, or 6 months after drug administration. Coupling was verified by vaginal smears; 50% of prenant females were killed by cervical dislocation on gestation day 20. The numbers of corpora lutea in the ovaries and implantation sites in the uterus were determined, and viable/dead fetuses were counted. The indices of preimplantation and postimplantation fetal death were calculated. The fetuses were removed and weighed, and their craniocaudal size was measured. Some fetuses were placed in Bouin's fixative for examination of internal organs by Wilson's method, and some fetuses were placed in 95% alcohol and stained according to Dawson's methods for visualisation of skeletal defects [2]. The remaining pregnant females were kept until labor,

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and physical development of the offspring was monitored for two months. The times of auricle detachment, appearance of primary hairs, incisor eruption, eye opening, testicular descent, and vaginal opening were recorded. The survival index was calculated on day 4 as the ratio of survivors to the total number of pups. Fetuses and newborn pups from intact females and males treated with cytostatics 1, 3, or 6 months before mating (or, vice versa, intact males mated to females receiving the same treatment) were analyzed.

In addition, we studied fetuses and pups from intact parents. The data were processed statistically using Student's *t* and Wilcoxon—Mann—Whitney tests, and Fisher's angular transformation.

RESULTS

Mating of intact rats with partners treated with anticancer drugs increased the indices of embryonic death (Table 1). The rate of preimplantation death was higher after administration of all anticancer drugs compared to the control, irrespective of the treatment-mating interval and the sex of treated partner. The strongest effect (a 4-fold increase in preimplantation death) was found after mating of intact females to males injected with Farmorubicin 1 month before fertilization. These data (with account of stages of spermatogenesis) suggest that Farmorubicin causes a stronger, in comparison to other drugs, toxic effect on spermatocytes. A 4-30-fold increase in the postimplantation death compared to the control was observed in females fertilized after cytostatic treatment. The highest postimplantation death was observed in females fertilized 1 month after administration of Doxorubicin and 3 or 6 months after administration of Platidiam.

Macroscopic examination of the offspring from test groups (2800 fetuses and pups) revealed four malformations (limb aplasia, fetus with two bodies and one head, and cerebral hernia). Macroscopic examination of the control groups (2880 fetuses and pups) found no apparent developmental anomalies. The incidence of spontaneous malformations in rats never exceeds 0.01%, *i.e.*, we found a 14-fold (0.14%) increase in this index after cytostatic treatment. The mean body weights and craniocaudal sizes of all fetuses were similar, except for fetuses obtained from intact females fertilized by males injected with Platidiam one month before mating (2.44±0.48 *vs.* 2.68± 0.05 g in the control, $p \leq 0.05$). In the test groups, the number of fetuses with hemorrhage in various organs and tissues, cholestasis, subcutaneous edema, adrenal hypertrophy, and fewer ossification sites was significantly increased compared to the control. A considerable retardation of skeleton ossification was observed in most fetuses from rats mated one month after cyto-

TABLE 1. Parameters of Offspring from Rats Injected with Anticancer Drugs at Various Terms before Mating to Intact Partners

Index	Treatment-mating interval, months																	
	1						3						6					
	females			males			females			males			females			males		
	D	F	P	D	F	P	D	F	P	D	F	P	D	F	P	D	F	P
Preimplantation death	+	+	–	–	++	–	–	–	+	–	–	+	–	+	–	–	–	–
Postimplantation death	++	–	–	–	–	–	+	–	++	–	–	–	+	+	++	–	–	–
External malformations	++	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Craniocaudal size	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Internal organs	+	+	–	+	–	+	–	+	–	–	–	–	–	+	–	–	+	–
Ossification	++	++	–	–	–	++	–	+	+	+	–	–	–	+	+	–	+	–
Survival rate	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Physical development	+	–	–	–	++	–	–	–	+	++	–	–	–	–	–	–	–	–

Note. D: Doxorubicin; F: Farmorubicin; P: Platidiam. Moderate effect (+), potent effect (++), no effect (—).

static treatment. Irrespective of the drug injected, sex of treated partner, and term of mating, the number of fetuses with pathologies in internal organs and retarded development of the skeleton was higher in test groups. A significant (more than 2-fold in comparison to the control) decrease in survival rate was found only in offspring of females treated with Doxorubicin. Considerable deviations in physical development (delayed eye opening and sexual maturation) were found in offspring of intact females mated to males treated with anthracyclin antibiotics Farmorubicin and Doxorubicin 1 and 3 months before mating, respectively.

Thus, treatment of either parent with equivalent doses of various anticancer drugs 1, 3, or 6 months before mating to intact partner caused fetal death and similar developmental disorders in survived offspring. Differences in the damage to the offspring produced by individual drugs were mainly found in the strength and time of appearance of their toxic effects. The decrease in fetal body weight and survival rate depended on the type of cytostatic drug, while the increase in postimplantation death depended on the sex of treated parent. The most pronounced changes were observed in offspring of females injected with Doxorubicin or Platidiam 1 and 3 months before mating, respectively. There were practically no disorders in offspring of intact females mated to males injected with Doxorubicin 6 months before mating. Anticancer drugs are chemical mutagens, therefore genetical determination of these toxic effects cannot be excluded. More considerable disorders in the offspring of females in test groups can be explained by higher sensitivity of female sex cells to drug-induced genotoxicity.

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