Status of the Progeny of Rats Treated with Platinum-Containing Cytostatics

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Wistar rat pups from rats treated with first- and second-generation platinum-containing cytostatics (platidiam, carboplatin) 1, 3, and 6 months before mating with intact partners had similar disorders. The severity of these disorders depended in many cases on the chemical structure of the drug and sex of the parent treated with the cytostatic. The severity of toxic effects in the progeny of intact females mated with cytostatic-treated males decreased with prolongation of the period elapsing between the treatment and mating. Carboplatin produced a more potent toxic effect on the reproductive function of rats compared to platidiam.

Key Words: *progeny; platidiam; carboplatin*

Encouraging results of therapy of some oncological diseases attracted the interest to the problem of sterility as an aftereffect of cytostatic treatment on actively proliferating tissues of reproductive organs [4,7]. Suppression of the reproductive function after treatment with some cytostatics is reversible. Moreover, children were born in families after one of the parents received cytostatic therapy [5,7,8]. Since antitumor drugs can induce cytogenetic injuries in sex cells [2], special attention is paid to the health status of children [6-8]. Antitumor drug therapy carried out in childhood had no serious impact on the status of the progeny [6]. Clinical data on the status of the progeny one of whose parents received cytostatics in the reproductive age are contradictory. Some authors claim that the incidence of congenital developmental defects is within the expected range [8], while others reported higher incidence of malformations, malignant tumors in children, and stillbirths [7]. Experimental studies showed that alkylating compounds (Thio-Tef), anthracyclin antibiotics (doxorubicin, farmorubicin) were toxic for the progeny [2,9,10]. Complex platinum compounds (CPC), the base of many treatment protocols occupying the leading place in modern chemotherapy of tumors, are less studied.

We evaluated the state of the progeny one of whose parents was treated with CPC 1, 3, or 6 months before mating with an intact partner.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old Wistar rats (360 females and 60 males, 250 g) from Laboratory of Biological Simulation, Institute of Pharmacology. A total of 3600 fetuses and 1200 rat pups were examined. Platidiam (first-generation platinum preparation, Lachema) and carboplatin (second-generation platinum preparation, ABIK Ltd.) were injected to rats intravenously in single maximum tolerable doses of 4 and 60 mg/kg, respectively. The doses were estimated by graphic probit analysis after 30-day observation. Control animals (120 females and 40 males) received no platinum preparations. One, 3, and 6 months after drug injection the animals were caged together with intact rats. Fertilization was verified by vaginal smears. Some pregnant females were sacrificed by cervical dislocation on day 20 of gestation and corpora lutea in the ovaries, implantation sites in the uterus, and live and dead fetuses were counted, after

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which the pre- and postimplantation mortality indexes were estimated. The fetuses were removed, weighed, craniocaudal size was measured, some fetuses were fixed in Bouin's fixative and then the viscera were examined by Wilson's method; other pups were fixed in 96% ethanol and stained by the method of Dowson for evaluation of skeleton abnormalities. The progeny of other pregnant females was examined during subsequent 2 months using the criteria for evaluation of the reproductive toxicity of drugs [3]. The survival index was estimated, general physical development of the progeny was monitored (dynamics of body weight gain, time of concha detachment, hair appearance, canine eruption, eye opening, and sexual maturing), the rate of maturing of the sensory and motor reflexes was evaluated on days 5, 15, and 30 of life by the turning over on the plane, precipice avoidance, muscular force, and open field tests [1]. Training capacity was studied in the passive avoidance paradigm (conditioned reflex) on day 60 of life.

The results were statistically processed using Wilcoxon—Mann—Whitney's test.

RESULTS

No significant increase in embryonic mortality in comparison with the control was observed in intact female rats mated with experimental males. Analysis of the data showed some common pathological changes characteristic of the progeny of intact females and males injected with CPC (Table 1): increased incidence of fetuses with pathological changes in the viscera, inhibited ossification processes ($p<0.05$), delayed physical development (delayed eruption of canines, low body weight), delayed formation of the sensory motor reflexes, muscle tone, and training capacity ($p<0.05$). The spectrum of disorders was more extensive in the progeny of intact females and males treated with carboplatin: in addition to the above-mentioned toxic effects, the survival index was low, horizontal and vertical motor activity in the open field test was increased ($p<0.05$). It is noteworthy that delayed ossification, pathological changes in the viscera, and delayed physical development were also observed in the progeny of male rats treated with cytostatics belonging to other groups, for example antitumor anthracycline antibiotics [2,9,10]. On the other hand, we know that administration of antitumor drugs belonging to other chemical classes to male mice can increase embryonic mortality. The absence of the increase in intrauterine mortality after treatment with platidiam and carboplatin confirm the data indicating that platinum-containing drugs cause no increase in the level of dominant lethal mutations in male sex cells [11].

Pathological changes in the progeny of intact females and treated males, induced by platinum-containing compounds, differed by the time of manifestation. Moreover, the intensity of manifestation of these shifts after injection of each drug was different in many cases. For example, ossification processes were inhibited

TABLE 1. Status of the Progeny of Rats Mated in Different Periods after Injection of Platinum Cytostatics ($M\pm m$)

Parameter	Males						Females					
	after 1 months		after 3 months		after 6 months		after 1 months		after 3 months		after 6 months	
	P	C	P	C	P	C	P	C	P	C	P	C
Preimplantation mortality	—	—	—	—	—	—	—	—	+	+	—	+
Postimplantation mortality	—	—	—	—	—	—	—	—	+	—	+	+
External abnormalities	—	—	—	—	—	—	+	—	—	—	—	—
Craniocaudal size	—	—	—	—	—	—	—	—	—	—	—	+
Pathological changes in viscera	++	—	—	+	—	+	—	++	—	+	—	+
Status of ossification processes	++	—	++	+	+	—	—	+	+	+	+	—
Survival index	—	+	—	—	—	—	—	—	—	++	—	—
Physical development	—	+	+	—	—	—	—	—	+	++	—	++
Muscle tone	+	+	—	—	+	—	+	—	+	—	—	—
Development of sensory motor reflexes	+	—	—	+	—	+	—	—	—	+	—	—
Behavior in the open field test	—	++	—	—	—	—	—	—	+	0	—	—
Training capacity	+	—	+	+	—	—	—	—	—	0	—	—

Note. P: platidiam; C: carboplatin; «—» no toxic effect for the tested parameter; «+» moderate, «++» high degree of toxic effect; 0: no progeny.

TABLE 2. Mortality of Fetuses of Female Rats Treated with Platinum-Containing Cytostatics before Mating (*M*±*m*)

Term of mating after drug injection		Preimplantation mortality, %		Postimplantation mortality, %	
		control	experiment	control	experiment
1 month	platidiam	2.5±1.6	11.2±2.6	1.3±0.9	4.9±2.1
	carboplatin	2.4±2.4	14.7±9.4	12.6±4.3	9.0±4.7
3 months					
	platidiam	2.5±1.6	29.2±6.3*	1.3±0.9	34.6±10.5*
	carboplatin	2.0±1.3	20.0±7.6*	11.0±4.8	17.9±6.9
6 months					
	platidiam	8.4±2.7	16.4±3.4	4.5±3.6	27.3±9.8*
	carboplatin	5.1±2.6	27.0±9.7*	6.7±2.3	37.2±11.8*

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

in the progeny of male rats treated with platidiam at all terms of observation and the incidence of pathological changes in the viscera was higher in the group mated one month after drug injection (Table 1).

Examination of the progeny of cytostatic-treated females showed a wider spectrum of disorders in comparison with the progeny of intact females and treated males. Increased embryonic mortality (Tables 1, 2) is worthy of note; it is in line with previous reports [11] that platinoid compounds increase the level of dominant lethal mutations in oocytes. Embryonic mortality was maximum 3 months after platidiam injection and 6 months after carboplatin injection (Table 2). Both indexes of embryonic death increased during these periods. The increase in the pre- and postimplantation mortality after injection of both drugs was similar. One case with malformation (a fetus with one head and two trunks, one of which had three limbs, the upper limb with 6 fingers) was detected in the progeny of female rats treated with platidiam. Abnormalities in the progeny of females treated with carboplatin were as follows: increased number of fetuses with visceral abnormalities at all terms of investigation and decreased craniocaudal size 6 months after the start of the experiment. A characteristic feature of the progeny in this group was an appreciable decrease (*vs*. the control) of body weight and low viability of rat pups. The survival index for the progeny of rats treated with carboplatin 3 months before mating was 0% by day 15 of life, which can be a result of toxic effect of the drug on maternal body.

The viability of the progeny of intact females and treated males mated 6 months after the start of the experiment was higher. The number of fetuses with visceral abnormalities was lower and the ossification processes were less inhibited in the progeny of males injected with platidiam 6 months before mating. The formation of the sensory and motor reflexes was not delayed in this group and training capacity was not impaired. After injection of carboplatin to males, such toxic effects as decreased survival index, slow physical development, decreased muscle tone, and increased horizontal motor activity were observed only in the progeny of animals mated 1 month after the cytostatic exposure. This regularity was not observed in evaluation of the status of the progeny of cytostatic-treated females, which was seen primarily from high embryonic mortality observed after mating 6 months after the treatment with both platidiam and carboplatin (Tables 1, 2).

Hence, the health status of the progeny of parents one of which was injected with platinum-containing drugs 1, 3, or 6 months before mating with intact partners was characterized by common disorders. The manifestation of some abnormalities depended on the chemical structure of the drug and sex of the parent treated with the cytostatic. The severity of toxic aftereffects in the progeny of males exposed to the cytostatics was lower in the groups mated after a longer period after drug injection. Since treatment of male rats with carboplatin resulted in manifestation of a wider spectrum of abnormalities in the progeny and treatment of females with this drug resulted in low viability of the progeny, we conclude that carboplatin is more hazardous for the reproductive function of rats than platidiam.

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