Evaluation of the Progeny of Rats Treated with Topoisomerase II Inhibitor Vepesid

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Progeny of Wistar rats treated with vepesid 1, 3, and 6 months before mating is characterized by common pathological changes. These changes were more pronounced and more diverse in animals descending from females receiving the cytostatic compared to the progeny of treated males. The severity of toxic effects depended on the period between mating and vepesid treatment. Cytostatic treatment 3 months before mating was associated with minimum toxicity for the progeny.

Key Words: *vepesid; toxicity; progeny*

Antitumor drug vepesid is widely used in oncology due to its wide spectrum of antineoplastic effects, capacity to enhance tumor sensitivity to other cytostatics, and possibility of use not only in injections, but also in capsules [3]. Vepesid is a topoisomerase inhibitor, but it is also known as an apoptosis inductor and a prooxidant [10,12]. Encouraging results in patients receiving chemotherapeutic schemes including vepesid necessitated evaluation of the risk of sterility as a possible aftereffect of this drug on actively proliferating tissues of male and female gonads. Clinical studies showed that spermatogenesis disorders and ovarian insufficiency observed during treatment with this drug were reversible [6,11], but sterility can be caused by its mutagenic effects. It was experimentally shown that vepesid induced cytogenetic injuries causing elimination of non-fertilized sex cells and formation of non-viable zygotes [5,9]. On the other hand, some authors described childbirth from parents one of whom had been previously treated with cytostatics, including vepesid [7,8], but the data on the status of the progeny are scanty. Taking into account the mechanisms of biological effect of this drug on cells we cannot exclude high risk of congenital abnormalities and hereditary diseases in the progeny.

We evaluated the status of rat progeny one of whose parents (father or mother) was treated with vepesid 1, 3, and 6 months before mating with an intact partner.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old Wistar rats (240 females and 120 males; 250 g). The animals were bred at Laboratory of Biological Simulation, Institute of Pharmacology, and were kept in accordance with the regulations of the European Convention for Protection of Vertebrates Used for Experimental and Other Research Purposes (Strasbourg, 1986). The status of progeny was evaluated on 917 fetuses and 417 rat pups. Vepesid (Teva) was intravenously injected to rats (males and females) in a single maximum tolerable dose (30.0 mg/kg) calculated by graphic probit analysis. Control rats (60 females and 30 males) received the solvent in an equivalent volume. One, three, and six months after drug injections the rats were caged together with intact animals, which corresponded to exposure of dividing epitheliocytes (males) and

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multilamellar and primordial follicles (females). Mating was confirmed by the analysis of vaginal smears. On day 20 of pregnancy some females were sacrificed by cervical dislocation and corpora lutea in the ovaries, implantation sites in the uterus, live and dead fetuses per female were counted. Indexes of pre- and postimplantation death were then estimated [4]. The fetuses were removed, weighed, examined visually, some of them were placed into Bouin's fluid for fixation and subsequent examination of the viscera by Wilson's method, other fetuses were fixed in 96% ethanol and stained by Dowson's method for evaluation of ossification processes [4]. Other females were observed until delivery. The status of the progeny was evaluated during 2 months after birth. The survival index was evaluated, physical development was evaluated by the dynamics of body weight gain, time of auricle separation, appearance of fur, eruption of canines, eye opening, and sexual maturation. On days 5, 15, and 30 of life maturity of sensory motor reflexes was evaluated by the "turning over on a plane", "edge avoidance", "muscular force", and "open field" tests. At the age of 60 days passive avoidance conditioning [4] and adaptive behavior in Hunderson's test modified by N. A. Bondarenko [1] were evaluated. The results were statistically processed using Mann—Whitney test and Fisher angular transformation.

RESULTS

Mating of animals treated with vepesid led to increased antenatal mortality. The percentage of preimplantation loss increased after treatment of males (mating 1 and 3 months after injection) and the percentage of postimplantation death increased after treatment of females (fertilization 6 months after injection; Table 1). Antenatal mortality increased to a greater extent in the progeny of females treated with vepesid (82.98±5.76 *vs.* 9.75±6.16% in control), while after treatment of males antenatal mortality was 25.45±5.16% (7.27±4.57% in control). High embryonic death can indicate induction of dominant lethal mutations (DLM) under the effect of vepesid in maturing male and female sex cells. This agrees with published data that vepesid increased the level of DLM in male mice [9].

Analysis of the status of viable progeny of experimental males and females showed similar toxic effects. The antenatal period was characterized by increased percentage of fetuses with visceral abnormalities and inhibited ossification; postnatal period was characterized by decreased muscular tone, rate of sensory motor reflexes formation (turning over on the plane and edge avoidance), disturbed orientation and exploratory behavior (open field test), impaired passive avoidance conditioning and adaptive behavior.

Analysis of the severity of these disorders in viable progeny in relation to the sex of the parent treated with the cytostatic showed higher incidence of toxic effects in fetuses and pups after treatment of the females. This regularity was most clearly seen in the analysis of visceral organs. For example, the number of fetuses with pathological changes increased by 23% in the progeny of females fertilized 1 month after vepesid injection and by 11% in the progeny of males (compared to the control). In addition to nephroptosis and pathological changes in the thymus detected in females of both experimental groups, the number of fetuses with hemorrhages, hydrocephalus, hemopericardium, and congestion in the liver increased in the progeny of females injected with vepesid. Disturbances in open field behavior, passive avoidance conditioning, and avoidance of stress situation were detected in the progeny of experimental females after mating at all periods after cytostatic treatment, but not in the progeny of male rats injected with vepesid.

In the progeny of female rats receiving the cytostatic we observed a wider spectrum of disorders than in progeny of males. Macroscopic examination of fetuses from experimental females showed 70-100% fetuses with external hemorrhages *vs.* 30-40% in control ($p<0.05$) and decrease in the survival index to 49.99% (*vs.* 95.83% in control, $p<0.05$). Eye opening was 2 days delayed in some rat pups. A male pup with significantly delayed sexual maturation was detected in the progeny of female rats injected with vepesid 1 month before mating; body weight of this pup at the age of 2 months was 38 g (180±10 g in control). Delayed sexual maturation, though less pronounced, was observed in other male pups of this group.

The spectrum of disorders and their severity were similar in the progeny of males mated 1 and 6 months after vepesid injection. The worst viability of the progeny of experimental females was observed after mating 6 months postinjection. The least number of pathological shifts in the progeny of both experimental groups was observed after mating 3 months following the treatment. This can indicate a lesser sensitivity of spermatogonias to the drug toxicity in comparison with spermatocytes and of primordial follicles in comparison with multilamellar. Hence, this mating period after vepesid injection is the optimal (for males and females) as regards its toxicity for the progeny.

TABLE 1. Progeny of Rats Mated at Different Periods after Vepesid Injection

Parameter	Period between mating and drug injection, months					
	1		3		6	
	males	females	males	females	males	females
Preimplantation mortality	+	—	—	—	+	—
Postimplantation mortality	—	—	—	—	—	++
External abnormalities	--	+	—	—	—	—
Visceral pathology	+	+++	++	++	+	+
Ossification status	+	++	++	+	+	++
External hemorrhages	—	+	—	+	—	+
Time course of pups' body weight increment	—	—	—	—	—	—
Survival index	—	—	—	—	—	+
Physical development	—	—	—	—	—	+
Muscular tone	+	+	—	—	+	++
Development of "turning over on plane" and "edge avoidance" reflexes	++	+	—	—	+	++
Open field behavior	+	+	—	++	++	+
Adaptive behavior	+	+	—	+	+	+
Passive avoidance conditioning	—	+	++	+	++	++

Note. "—": no toxic effect; "+": mild toxic effect; "++": moderate toxic effect; "+++": pronounced toxic effect.

The detected pathological changes in fetuses and pups one of whose parents was injected with vepesid are not specific and are described (in similar experiments) for the progeny of rats injected with antitumor drugs with other mechanism of action [2], but the viability of the progeny as a rule increased with prolongation of the period from cytostatic treatment to mating. High antenatal mortality, high incidence of pathological changes in pups born after mating in the most delayed periods after vepesid injection seem to result from suppression of intracellular reparation systems in spermatogonias and in primordial follicular oocytes. A significant decrease in the viability of experimental female rats can be due to greater sensitivity of female gametes in comparison with the male ones to the toxic effect of this drug. Delayed toxic effects of vepesid, manifesting in female rats during pregnancy, cannot be ruled out.

Hence, the progeny of rats one of whose parents was treated with vepesid 1, 3, and 6 months before mating, was characterized by low viability. The severity of toxic effects depended on the sex of the parent treated with the drug and the time of mating after treatment. Vepesid exhibited a more pronounced toxic effect on the progeny of females injected with this drug. The optimal period for mating, with regard to the least toxicity for the progeny, is 3 months after cytostatic treatment.

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