Effect of Vepeside on the Function of Reproductive System in Rats

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Experiments on adult rats showed that a single intravenous injection of antitumor drug vepeside in a MTD (maximum tolerable dose) reduced the reproductive status during periods corresponding to exposure of mature sex cells, spermatocytes, and spermatogonia in male rats and exposure of oocytes in ovulating, mature, and primordial follicles in female rats. Reduction of the male and female reproductive function manifested in increased antenatal mortality of the progeny. The toxic effects of the drug on mature male sex cells caused temporary partial infertility.

Key Words: *vepeside; reproductive system; rats*

Antitumor drug vepeside (topoisomerase inhibitior) is used with good results for the treatment of testicular tumors, small-cell pulmonary carcinoma, trophoblastic tumor, and Hodgkin's disease. Chemotherapy of tumors of these location leads to remission in a high percentage of cases [4]. The probability of loss of the reproductive capacity because of high sensitivity of gonads to cytostatic treatment explains the interest of clinicians to studies of the reproductive status of male and female patients treated with vepeside [7,8]. Clinical data indicate that the incidence of infertility in patients receiving this drug varies. The number of experimental reports on gonadotoxic effects of vepeside increased in recent time. The interest to this problem is also associated with investigation of the role of DNA topoisomerases in meiosis and spermatogenesis processes. It was shown that cytogenetic disorders caused by vepeside can lead to elimination of maturing male sex cells and the formation of nonviable zygotes [5,9,10]. The data on the reproductive status of animals treated with vepeside during periods corresponding to spermatogenesis stages are scanty. The female reproductive status during vepeside treatment is also little studied.

We evaluated the reproductive function of adult rats treated with vepeside during periods corresponding to different stages of gamete formation. High-dose therapy is used in patients with favorable prognosis [4,7], and hence, the drug was injected in a single MTD.

MATERIALS AND METHODS

Experiments were carried out on 430 outbred male and female albino rats (250-300 g) from Laboratory of Experimental Biomedical Simulation, Institute of Pharmacology. Some rats were controls. In addition to these animals, 320 rat pups (first-generation progeny; one of the parents received vepeside) and 137 control rat pups (first-generation progeny; one of the parents received the solvent) were examined. The animals were kept in accordance with the regulations of the European Convention for Protection of Vertebrates Used for Experimental and Other Research Purposes (Strasbourg, 1986). Vepeside (etoposide, Tewa) was injected in a single MTD (30 mg/kg); the dose was calculated by graphic probit analysis after 30-day observation of animals. Control rats and intact partners received no vepeside (n=220).

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The reproductive function of rats was evaluated by the ability of mating, efficiency of mating, dynamics of fetal mortality, and progeny survival index. The animals were mated during periods corresponding to stages of the formation of female and male sex cells. Experimental and control males (10 per group) were placed together with intact females (in 1:2 ratio) on days 1-7, 16-22, 36-42, 90-96, and 180-186 after the drug injection (exposure of spermatozoa, spermatides, spermatogonia) [3]. Experimental and control females (20 per group) were caged together with intact males on days 1-10, 30-40, 90-100, and 180-190 after the start of the experiment (effects exposure of ovulating and mature, bi- and monolamellar, primordial follicles) [3]. Mating was evaluated by vaginal smears. Mating capacity was determined as the ratio of fertilized females to the number of females placed together with males; mating efficiency was determined as the ratio of pregnant to fertilized females. On day 20 of pregnancy the females were sacrificed, autopsied, and corpora lutea in the ovaries, implantation sites in the uterus, and numbers of live and dead fetuses per female were counted. The indexes of pre- and postimplantation mortality were calculated [2]. The status of the progeny from animals mated in delayed periods (30, 90, and 180 days) after vepeside treatment was evaluated. To this end, some pregnant females were left until delivery, the day of delivery was fixed, macroscopic examination of fetuses was carried out, and the survival index was estimated (the ratio of rat pups surviving until day 21 to the number of live-born rat pups).

The results were statistically processed using Wilcoxon—Mann—Whitney test and Fisher's angular transformation.

RESULTS

Study of the integral parameter characterizing the reproductive function of animals showed that the fertility index in males and females treated with vepeside virtually did not decrease in comparison with the control during all periods of observation (Fig. 1, *a*, *b*). Mating of intact females with experimental males on days 1-7 after the start of the experiment (exposure of mature cells) showed a decrease in the pregnancy index (by 46% of control; Fig. 1, *a*). Impairment of nonproliferating cells under the effect of vepeside was demonstrated in studies of the drug effect on the erythron [1]. This toxic effect can be due to the drug capacity to accelerate lipid peroxidation and lead to impairment of cell membrane integrity [12]. Apoptosis-inducing effect of vepeside on the spermatogenic tissue cells was shown [12]. The efficiency of mating during periods (days 36-42, 90-96, 180-186) corresponding to exposure of epitheliocytes with high level of topoisomerase activity (meiotically dividing spermatocytes, mitotically dividing spermatogonia) was not reduced. Pregnancy indexes in experimental females virtually did not decrease in comparison with the control throughout the entire experiment (Fig. 1, *b*).

Administration of vepeside to males and females increased antenatal mortality in the progeny (Fig. 2). Injection of vepeside to male rats (Fig. 2, *a*) increased preimplantation, while treatment of the females increased postimplantation fetal death (Fig. 2, *b*). Fetal death in intact females mated with experimental males increased in comparison with the control values after mating on days 36-42 and 180-186 of the experiment (exposure of cells with increased topoisomerase activity), which seems to be a result of vepeside induction

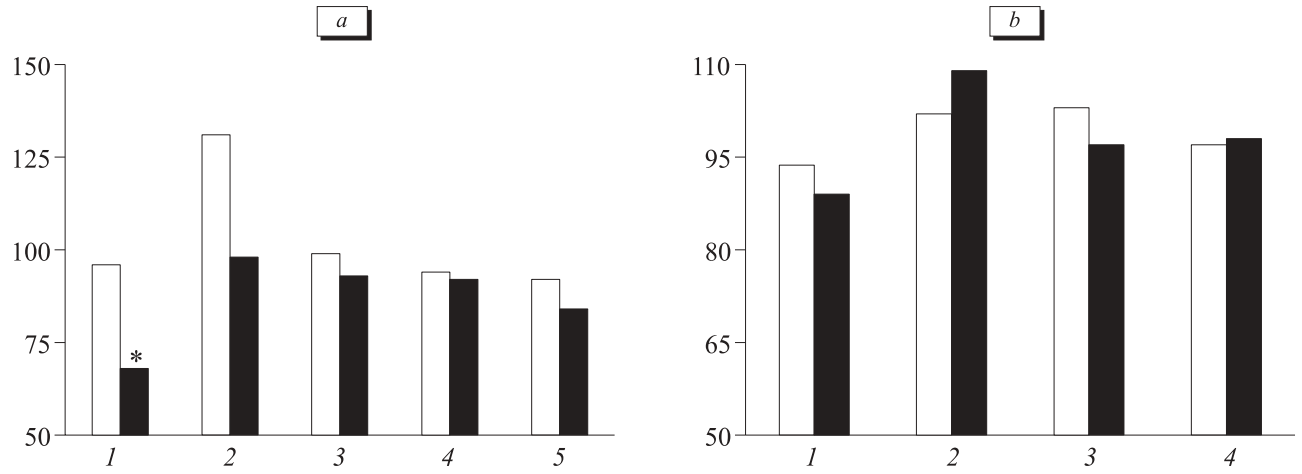

Fig. 1. Fertility (light bars) and pregnancy indexes (dark bars) of male and female rats mated with intact animals in different periods after injection of vepeside (% of control). Here and in Fig. 2: *a*) mating of experimental males with intact females: days 1-7 (*1*), 16-22 (*2*), 36-42 (*3*), 90-96 (*4*), 180-186 (*5*); *b*) mating of intact males with experimental females: days 1-10 (*1*), 30-40 (*2*), 90-100 (*3*), 180-190 (*4*) after vepeside injection. *$p<0.05$ compared to control (taken for 100%).

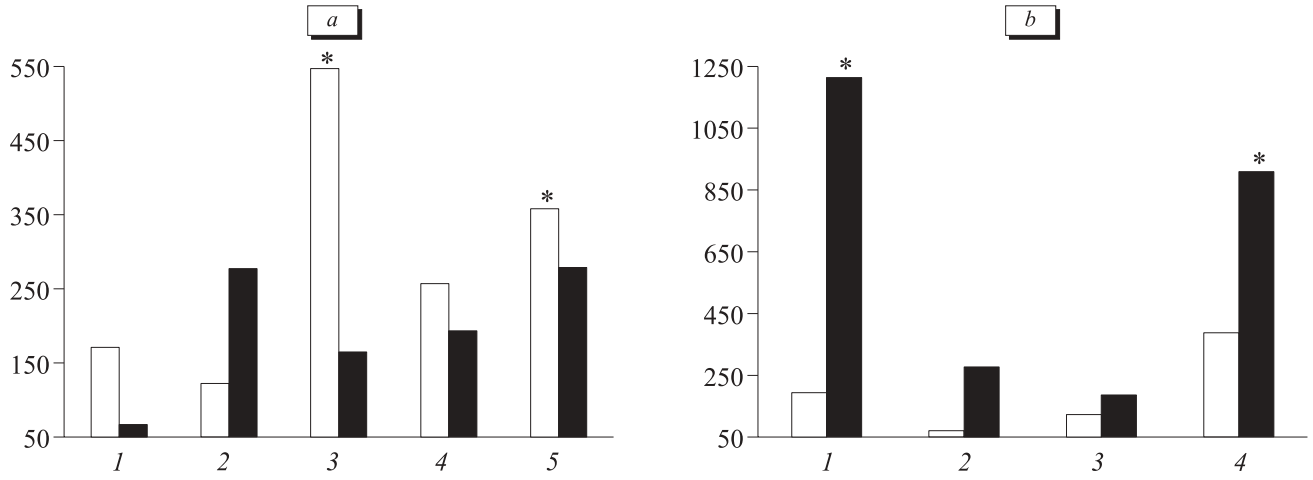

Fig. 2. Preimplantation (light bars) and postimplantation mortality (dark bars) of the progeny of male and female rats mated during different periods after vepeside injection (% of control).

of dominant lethal mutations (DLM) in spermatocytes and stem spermatogonias. The capacity of the drug to increase the level of DLM in mouse male gametes was demonstrated previously [9]. This toxic effect was not observed on days 90-96 of the experiment, presumably because the drug did not increase DLM at the stage of differentiated spermatogonias, though these cells are also targets for topoisomerase inhibitor [10]. An appreciable increase of fetal mortality in female rats was detected during periods corresponding to exposure of ovulating and mature oocytes (days 1-10) and primordial folliculi (days 180-190; Fig. 2, *b*), which can result from vepeside induction of DLM in oocytes. Lethal mutations are quantitatively less significant for the genesis of stillbirths than disorders in normal gestation [6], and we therefore have to admit that high implantation mortality in the progeny of females treated with vepeside can be a result of delayed toxic effect of vepeside on maternal organism.

Macroscopic examination of fetuses and rat pups of all experimental groups showed no visible developmental abnormalities. The study of the postnatal deaths showed that survival indexes tended to decrease in some periods of observation, but the differences were statistically insignificant.

Hence, the reproductive function of rats depended on the period of mating after vepeside treatment. Reduction of the reproductive function of male rats was caused by toxic effect of the drug on spermatozoa, spermatocytes, spermatogonia; in female rats the reduction of the reproductive function was caused by exposure of oocytes in ovulating, mature, and primordial folliculi. Suppression in male and female reproductive functions manifested in increased antenatal mortality of the progeny. Temporary partial sterility was observed in the males, indicating a greater sensitivity of the male reproductive function (in comparison with the female system) to the toxic effect of vepeside.

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