REVIEWS

Gonadotoxic Effects of Antitumor Preparations

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Here we review the results of experimental and clinical observations concerning the gonadotoxic effect of antitumor chemotherapy. Previous experiments showed that antiblastic preparations with various mechanisms of cytostatic action produce damage to the sex glands that differs in the severity, stage of manifestation, and period of reparative regeneration. These differences are related to different sensitivity of epitheliocytes in the testes and structural and functional elements in the ovaries. Morphological changes in the gonads are accompanied by dysfunction of the reproductive system. We compared damages to the reproductive system and its individual components produced by various antitumor preparations. The offspring was examined after cytostatic treatment of one of the parent animals.

Key Words: *antitumor preparations; toxicity; gonads; reproductive system; offspring*

Sex glands are intensively proliferating cell systems. These glands, blood system, and gastrointestinal mucosa are first exposed to the toxic effect of antitumor preparations [15,35]. Repeated mitotic divisions of spermatogonia and 2 mitotic divisions of primary and secondary spermatocytes lead to maturation of male sex cells in the testes. The structure and functional state of the ovaries with cyclic activity undergo constant variations [10,29].

Increasing interest to the problem of sterility resulting from the influence of cytostatics is associated with considerable advances in antitumor therapy [34, 38,41]. Drug therapy of some malignant neoplasms is followed by prolonged remission [27]. The number of patients with early stages of cancer increases due to the use of modern diagnostic methods in oncology. These patients are highly sensitive to medicinal treatment, including chemotherapy. It is important to preserve the quality of life in patients, particularly, taking into account their young age. Side effects of antiblastic preparations on spermatogenesis and ovarian function are of considerable biological, social, and psychological importance. Therefore, the gonadotoxic influence of these preparations requires detailed investigations [34]. Clinical observations indicate that cytostatic chemotherapy affects reproductive function [38, 41,50]. The search for new approaches to the correction of complications resulting from chemotherapy is an urgent problem [34].

Antitumor preparations produce a strong toxic effect on the sex glands, which determines the necessity of detailed experimental studies. Until recently, only the gonadotoxic action of alkyl compounds (AC) was studied [1,25,30]. Our experimental studies performed over several years were directed toward evaluation of changes in the reproductive system produced by various antitumor agents, including complex mercury compounds (platidiam and carboplatin), anthracycline antibiotics (AA; doxorubicin and farmorubicin), and plant preparations (vepeside). To achieve maximum effect, modern antitumor therapy follows the principle of chemotherapeutic radicalism [27]. Antiblastic preparations were administered once in maximum permissible doses (MPD). The gonadotoxic effects of these major cytostatic agents [11,14, 24,28] are poorly understood or explained contradictorily [46,47]. The type, severity, dynamics, and mecha-

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nisms of reproductive disorders and peculiar gonadotoxic effects of various preparations were evaluated in our previous experiments. Clinical trials with these preparations are difficult, because patients with tumors usually receive combination chemotherapy. It should be emphasized that studies of the reproductive system in experimental animals allows us to perform detailed morphometric examination of the sex glands, determine individual functional parameters and integral characteristics of this system (copulatory activity and efficiency), and evaluate the state of offspring.

Male Reproductive System during Cytostatic Disease

The results of morphological assay and published data indicate that antitumor preparations belonging to various chemical classes (AC, thiophosphamide; synthetic preparations, procarbazine; antitumor antibiotics, olivomycin, rubomycin, adriamycin, and farmorubicin; platinum-containing preparations (PCP), platidiam and carboplatin; and plant preparations, vincristine, Taxol, vepeside, and camptothecin) produce similar morphological changes in the male sex glands in mice, rats, and dogs. These changes include interstitial edema, vasodilation, and dystrophic and destructive processes in epitheliocytes [3,8,9,17,20,25, 31,33,36,57].

The severity of damage depends on the dose of preparations. PCP, AA, and podophyllotoxin derivatives in MPD produce reversible damage to the testes in Wistar rats. In animals receiving AA morphological picture returns to normal later than after treatment with other preparations. Vepeside, vinblastine, and farmorubicin cause the appearance of giant spermatogenic cells [17,20,55]. Changes in interstitial endocrine cells manifesting in a decrease in their number and size were found in the male sex glands of animals receiving platidiam, doxorubicin, and vincristine [3, 22,36]. Platidiam and vincristine in toxic doses produce damage to sustentocytes [36,51].

Antitumor preparations belonging to various chemical classes cause different toxic effects on the testes. The rate of regenerative processes after treatment with various preparations is different. Therefore, it is possible to perform a quantitative analysis of morphological disturbances. Treatment with platidiam, carboplatin, farmorubicin, and vepeside increased the number of seminiferous tubules with desquamed epithelium (Fig. 1). These changes were most pronounced after administration of PCP (carboplatin). Intensive deepithelization was observed at various stages of spermatogenesis. These data suggest that the drug not only produces a direct adverse effect on spermatogenic tissues, but also causes the appearance of pathological cells and their elimination. PCP produce damage to epitheliocytes of various types, which is probably associated with phase-nonspecific effects of these preparations.

Antiblastic preparations suppress proliferative activity to a different extent (Fig. 1). Seminiferous tubules with stage 12 meiosis disappear over the first few days after administration of podophyllotoxin derivative that binds to tubulin and suppresses the formation of mitotic spindles. Platidiam is the only PCP that significantly reduces this parameter. Meiotic activity of secondary spermatocytes in the testes of animals receiving AA was suppressed at later stages. Taking into account the duration of spermatogenesis in rats, it can be suggested that these changes were associated with exhaustion of the pool of proliferating spermatogonia due to the toxic effect on primary spermatocytes.

The index of spermatogenesis determined by the number of layers in spermatogenic tissues markedly decreased after administration of vepeside (Fig. 1), which is probably related to inhibition of meiotic activity. Thinning of the spermatogenic tissue in animals treated with platidiam and farmorubicin was less pronounced. Carboplatin did not decrease this parameter.

The count of spermatogonia decreased over the first days after administration of carboplatin (Fig. 1). Platidiam reduced the number of spermatogonia at later stages. However, these changes were less pronounced than after treatment with other cytostatics. Farmorubicin progressively decreased the number of spermatogonia in the testes of animals, which reflects damage to stem cells. This process probably delays reparative regeneration. Selective damage to spermatogonia is produced not only by farmorubicin. Preparations of this group (doxorubicin) [48] and antiblastic drugs with another mechanism of action (thiophosphamide) induce similar changes [1]. The biological effects of AA are associated with the ability to impair DNA synthesis. During spermatogenesis DNA is duplicated in spermatogonia and primary spermatocytes (preleptotene stage). Therefore, these cells are the main target for the toxic effect of AA.

Clinical observations indicate that cytostatic chemotherapy is followed by oligospermia or azoospermia, which leads to sterility due to the inhibition of spermatogenesis [15]. The total number of sex cells in the epididymis of experimental animals (rats) was determined during spermatogenesis. Platidiam, carboplatin, doxorubicin, farmorubicin, and vepeside in MPD decreased the number of sex cells over the first days after treatment. Therefore, these preparations produce damage to spermatozoa. Toxicity of preparations decreased in the following order: AA→(farmorubicin→doxorubicin)→podophyllotoxin derivatives (vepeside)→PCP (carboplatin→platidiam). The total

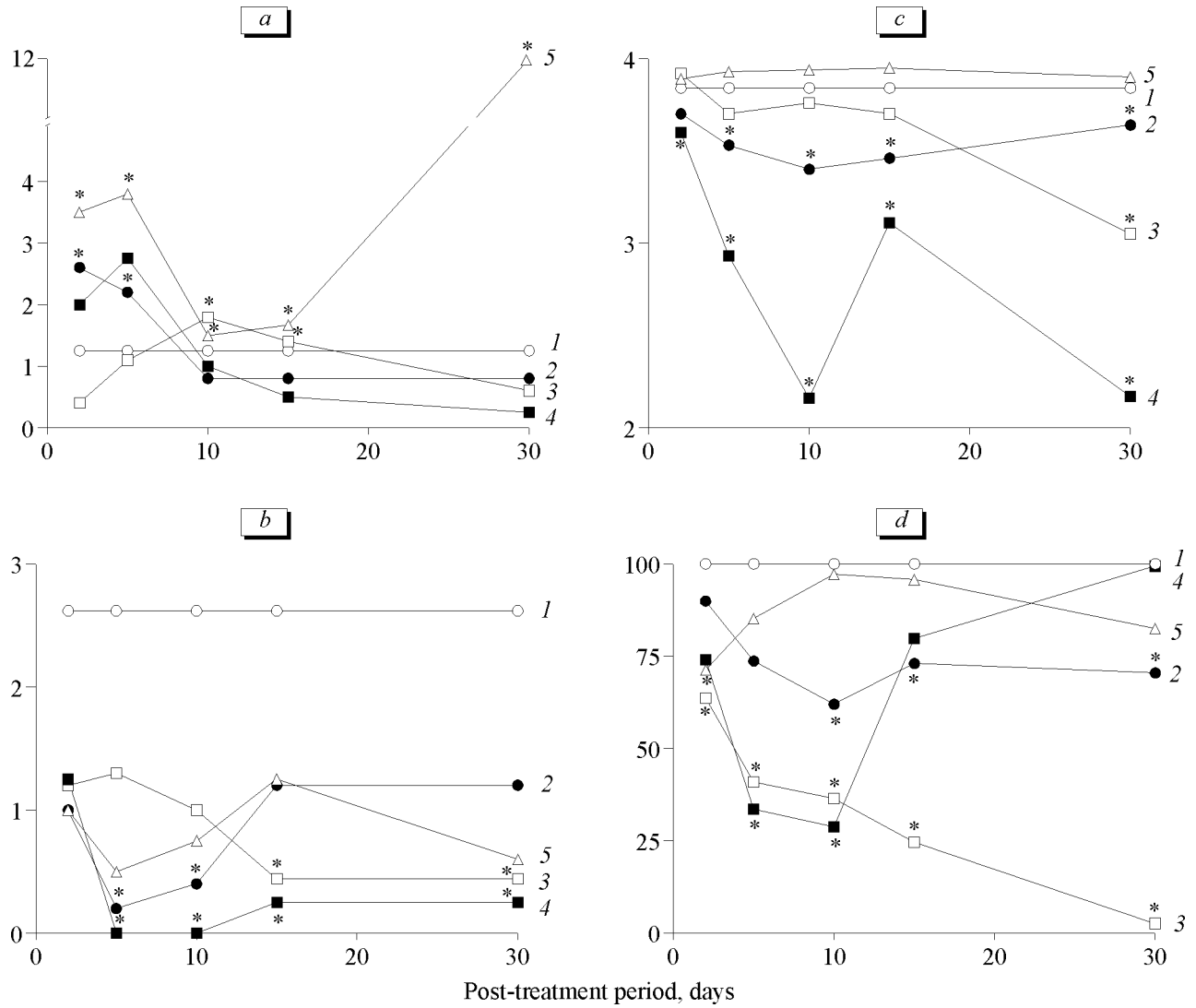

Fig. 1. Morphological parameters for generative function of rat testes after single treatment with antitumor preparations in maximum permissible doses: tubules with desquamed epithelium (%, *a*), tubules with stage 12 meiosis (%, *b*), index of spermatogenesis (arb. units, *c*), and number of normal spermatogonia (% of the control, *d*). Control (*1*), platidiam (*2*), farmorubicin (*3*), vepeside (*4*), and carboplatin (*5*). *$p<0.05$ compared to the control.

number of sex cells decreased in the late period after administration of doxorubicin, farmorubicin, and carboplatin. These data, duration of spermatogenesis in rats, and period for manifestation of oligospermia suggest the development of toxic changes in spermatocytes (doxorubicin, carboplatin, and vepeside) and spermatogonia (AA) [6,8,9,17,18,20,22]. It should be emphasized that morphological changes in the testes and abnormal maturation of male sex cells was accompanied by ineffective copulation only after treatment with farmorubicin and carboplatin [22]. The development of sterility in animals receiving AA coincided with pronounced oligospermia induced by these preparations. Carboplatin decreased copulatory efficiency due to functional inactivation of spermatozoa. This preparation decreased the number of motile spermatogonia and maximum duration of motility [8, 22]. This impairment of reproductive ability was reversible and related to the toxic effect on spermatozoa, spermatogonia (farmorubicin), and spermatids (carboplatin) [22].

An integral parameters characterizing generative activity of the testes in experimental animals is the prenatal mortality rate in mated females. A decrease in the number of survived zygotes reflects functional (genetic) incompetence of spermatozoa. The prenatal mortality rate increases in female mice and rats mated to males receiving AC [26], PCP [22], and AA [9].

Clinical observations indicate that antitumor preparations produce a systemic effect, inhibit spermatogenesis, and decrease potency and libido [54]. Studies of copulatory ability after treatment with platidiam, carboplatin, doxorubicin, and farmorubicin in MPD showed that sexual activity decreased only in Wistar rats receiving AA [22].

Female Reproductive System during Cytostatic Disease

Morphological examination of the female sex glands after long-term chemotherapy with AC revealed only stromal cells [15]. Antitumor preparations of various chemical classes produce similar morphological changes in the ovaries of experimental animals that depend on the dose of these agents. These changes include interstitial edema, death of cells in the follicular epithelium, degeneration of the nuclei in eggs, and formation of cysts in follicles and corpora luteum [1,19,21,22,25,30]. Counting of structural-and-functional elements in the ovaries allows us to evaluate peculiar effects of each preparation and perform a comparative quantitative analysis of damage. Clinical trials with these preparations are difficult. Serial slices of the ovaries of estrus rats receiving AA and PCP in MPD (farmorubicin and platidiam, respectively) were subjected to histological assay [19,21,22]. It was shown that primordial follicles are the target for direct toxic action of farmorubicin (Fig. 2). The number of multilayer follicles decreased over the first days after treatment with platidiam. These changes were followed by a decrease in the total number of generative cells. As differentiated from farmorubicin, platidiam did not increase the count of atresic follicles (Fig. 2). PCP and AA decreased the number of generative cells, which was followed by early exhaustion of reserve capacities in the ovaries. These changes were most pronounced after administration of AA (Fig. 2). Test preparations did not decrease the count of mature follicles and corpora lutea [19,21,22]. We compared the results of quantitative studies with published data [1, 30,43]. PCP (platidiam) and AA (farmorubicin) produce less significant morphological changes in the ovaries than antimetabolites (methotrexate) and AC (thiophosphamide and cyclophosphane).

Morphological changes in the female sex glands produced by cytostatic chemotherapy were accompanied by impairment of hormonal activity. For example, women receiving cytostatic chemotherapy often suffer from menstrual dysfunction and hypergonadotropic hypogonadism [15,34]. The incidence of hormonal disorders depends on the age of patients and type of chemotherapy. These disturbances most often accompany AC treatment. PCP (platidiam and carboplatin) and AA (farmorubicin) increase the duration of estrus cycle due to lengthening of diestrus [19,21,22], which reflects suppression of hormonal activity in the ovaries. The intensity of estrogen secretion peaks by the end of diestrus and during proestrus, which coincides with intensive growth of follicles. The decrease in the number of follicles revealed during morphological assay can contribute to lengthening of diestrus. Experiments of G. A. Savitskii *et al.* (1977) showed that antimetabolite methotrexate in therapeutic doses prolong diestrus in rats [30]. Lengthening of diestrus in methotrexate-receiving animals was more pronounced than after treatment with PCP and AA, which caused prolonged sterility.

Platidiam, carboplatin, and farmorubicin impaired the copulation ability for a short time and had no effect on the index of animal's fertility. We evaluated copulatory efficiency and prenatal mortality rate in rats receiving platidiam, carboplatin, doxorubicin, and farmorubicin in stages corresponding to maturation of follicles. Pregnancy developed only in 28-57% fertilized females, in whom the drug acted on preovulatory follicles (oocytes in active phase of meiosis) [7,22]. The index of pregnancy was lowest after administration of doxorubicin. This preparation caused sterility in females mated in the period of action on primordial follicles (oocytes in the dictyotene stage of meiosis I). Test preparations affecting primordial, multilayer, and preovulatory follicles increased prenatal mortality rate to 18-72% compared to the control [4,7,18]. By the inhibition of reproductive functions, toxicity of preparations decreased in the following order: doxorubicin→farmorubicin→carboplatin→platidiam. Functional disturbances in the female reproductive system produced by antiblastic preparations probably result from damage not only to the sex glands, but also to other organs and the whole maternal organism [22].

Our results and published data indicate that antitumor preparations possess pronounced gonadotoxic activity independently on the mechanism of their cytostatic action. Both dividing and non-dividing cells of the sex glands are highly sensitive to the cytostatic effect of these agents. The formation of damage to non-proliferating cells is probably related to the ability of antiblastic preparations produce the toxic effect on cell membranes, induce free radical generation, and initiate apoptosis. The influence of antitumor preparations belonging to various chemical classes differs in the severity of gonadotoxic damage, stage of manifestation, and period of reparative regeneration. Probably, various phases of the cell cycle and structural and functional elements of the sex glands are characterized by different sensitivity to the gonadotoxic effect of these preparations.

Experimental and clinical investigations are directed toward reduction of gonadotoxic effects of cytostatic chemotherapy [34,40,42]. A decrease in the number of potential target cells by suppression of proliferative and hormonal activity in the ovaries is an approach to the protection of female sex glands. Estrogen-gestagen preparations are used in clinical practice for this purpose [34]. Experiments on rats and dogs showed that testosterone and luliberin antagonist can

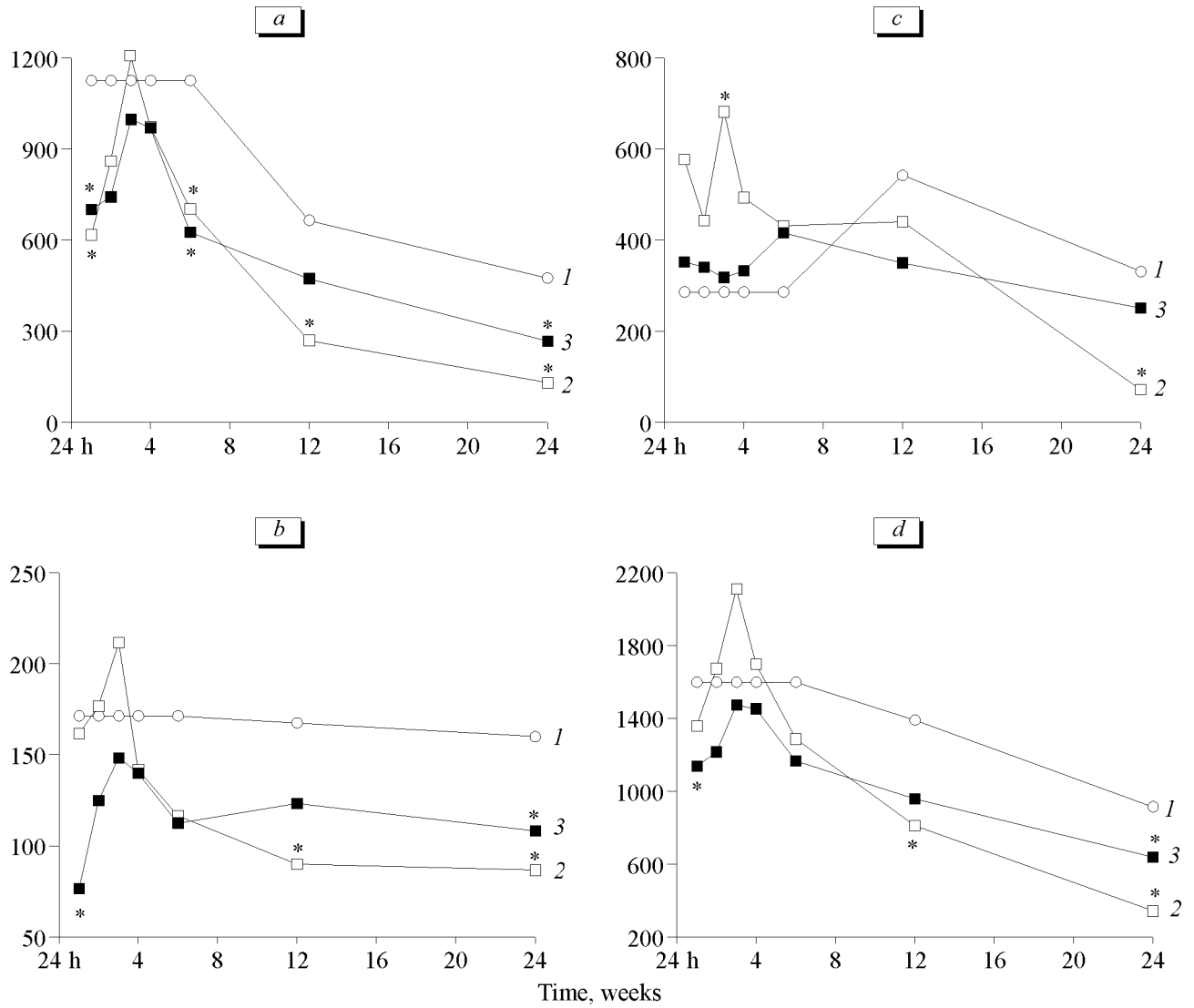

Fig. 2. Structural and functional elements of rat ovaries in the control (*1*) and after administration of farmorubicin (*2*) and platidiam (*3*): rectangular follicles (*a*), bi- and multilayer follicles (*b*), atresic follicles (*c*), and total number of generative cells (*d*). *$p<0.05$ compared to the control.

stimulate spermatogenesis [40,42]. Studies of the gonadotoxic activity in antiblastic preparations indicate that stimulation of spermatogenesis is important during treatment with cytostatics producing selective damage to spermatogonia (AC and AA). Ovary-protecting preparations should be used during therapy with AC, antimetabolites, and AA.

Effect of Antitumor Preparations on the Offspring

Much attention is given to the stage of offspring after cytostatic treatment of one of the parent animals. On the one hand, it is related to success in drug therapy of various malignant neoplasms. On the other hand, published data show that antiblastic preparations do not cause cytogenetic damage to cells in normal (nontumor) tissues (including gonads). Cytostatic factors increase the incidence of dominant lethal mutations [26,58], receptor translocations [49], and aneuploid spermatocytes [33], which reflects the formation of damage causing fetal death and appearance of genetically abnormal offspring. Many cases of childbirth in patients with long-term complete remission after cytostatic therapy of lymphogranulomatosis, breast cancer, testicular cancer, cancer of the prostate, trophoblastic tumor, and germ cell tumor of the ovaries were reported [37,39,44,45,50,52]. There are contradictory data on the state of children from parents receiving antitumor therapy. Clinical observations of S. Retsas *et al.* (1996) showed that the incidence of abnormalities in these children is within expectable limits. Stillbirths, malformations, and increased incidence of malignant neoplasms were reported by other authors [37,50]. The offspring of animals treated with cytostatic preparations before mating was extensively

studied [4,5,7,18,23,30,53,56]. G. A. Savitskii *et al.*, (1977) did not reveal serious abnormalities in the development of offspring from female rats receiving antimetabolites (methotrexate) in a therapeutic dose [30]. Pretreatment of experimental animals with AA increases prenatal mortality rate, causes developmental abnormalities in the offspring, decreases the weight of fetuses, and impairs skeleton formation [53,56].

We examined the offspring of Wistar rats after single administration of PCP (platidiam and carboplatin) and AA (doxorubicin and farmorubicin) in MPD to the male or female parent [4,5,7,18,22,23]. Experimental animals were mated with intact partners in the delayed period after cytostatic treatment (1, 3, and 6 months). To study the effect of antiblastic preparations on the offspring we determined parameters of fetal death, performed macroscopic examination of fetuses, evaluated the state of internal organs and skeleton, and recorded survival rate and physical development of rat pups.

Test cytostatics increased prenatal mortality rate to 53% (*vs.* 10% in the control) independently on the period of mating and gender of a parent animal receiving the preparation. However, mortality rate increased to a different extent after administration of various preparations. It depended on the class of administered chemical compounds. AA more significantly increased the prenatal mortality rate than PCP. The chemical composition of preparations also affected the prenatal mortality rate. Doxorubicin and platidiam were more toxic than farmorubicin and carboplatin, respectively. Moreover, the prenatal mortality rate was highest in cytostatic-receiving females. The prenatal mortality rate did not decrease with lengthening of the interval between treatment and mating. Macroscopic examination revealed malformations in 4 of 3500 fetuses and rat pups: 1 specimen with 1 head and 2 bodies (administration of platidiam to females 1 month before mating), 2 rat pups with aplasia of extremities (administration of doxorubicin to females 1 month before mating), and 1 rat with hernia of the brain (administration of farmorubicin to males 6 months before mating). It should be emphasized that the incidence of spontaneous malformations in rats does not exceed 0.01%. Our results show that the incidence of spontaneous malformations in the offspring increased by 14 times (0.14%) after cytostatic treatment of one of the parent animals.

The average weight and craniocaudal size were similar in experimental and control rats. We examined internal organs and skeleton in rats. The rats receiving antiblastic preparations produced a greater number of fetuses with hemorrhages in various organs and tissues, cholestasis, edema of subcutaneous fat, increased adrenal glands, and low number of ossification points of the skeleton. These cytotoxic effects did not depend on the type of cytostatic exposure, gender of parent animals receiving the preparation, and period of mating. However, the ratio of fetuses with internal disorders was higher in the offspring of females treated with antitumor preparations. The survival index decreased only in the offspring of females receiving doxorubicin and carboplatin. These changes were most pronounced after treatment with carboplatin 3 months before mating. It should be emphasized that lengthening of the period between treatment and mating increased the number of survived rat pups. The impairment of physical development (delay in eye opening and sexual maturation) was observed only in the offspring of males and females receiving AA 1 and 3 months before mating, but not 6 months before mating.

These data indicate that antitumor preparations of various classes produce toxic effects on the offspring of male and female animals. Some fetuses died. Pathological changes in survived animals were similar. The toxic effect of test preparations on the offspring differed in the degree and stage of manifestation. Doxorubicin and carboplatin produced most serious damage to the offspring. It should be emphasized that pathological changes were most severe in the offspring of females receiving antitumor preparations. It was probably related to higher sensitivity of female sex cells to the genotoxic effect of preparations. Moreover, antiblastic preparations primarily produce damage to the maternal organisms. In our experiments female rats were mated in the delayed period after cytostatic treatment. However, these animals were characterized by morphological and functional insufficiency of systems responsible for fetal development. Published data show that cytostatic treatment caused delayed consequences in nontumor tissues [13,16]. The offspring of animals mated 6 months after cytostatic treatment was characterized by a higher survival rate and normal physical development.

The results of studying gonadotoxic effects produced by antitumor preparations in experimental animals can be extrapolated to humans. However, this approach is associated with some problems. Experimental animals are characterized by multifetal gestations and, therefore, the parameter "partial fetal death" is not absolutely adequate. Taking into account the hypothesis of I. V. Vorobtsova [12], it can be suggested that cytostatic treatment in humans will be followed by a higher rate of prenatal death during unidentified pregnancy and lower incidence of serious abnormalities in the offspring.

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