EFFECT OF ANTENATAL EXPOSURE TO THIOTEPA ON SPERMATOGENESIS OF MATURE 101/H AND CBA MICE

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The etiology of development of pathological changes, including chromosome diseases, in the germ cells, the problem of the degree of their sensitivity and vulnerability at different stages of gametogenesis, and analysis of the late consequences of prenatal exposure of the developing gametes to the action of harmful agents has not yet drawn sufficient attention in the special literature.

The results of a study of interlinear differences among laboratory mice with regard to the regenerative capacity of the germ cell pool after exposure to therapeutic substances used in medical practice may provide a basis for the further study and understanding of the mechanisms of genetic control of sensitivity of mammals to the gametotoxic action of exogenous factors.

The aim of the present investigation was to study differences in the degree of disturbance and recovery of spermatogenesis in the mature offspring of different strains of mice after antenatal exposure to thiotepa, an alkylating agent which induces chromosomal aberrations in the somatic and spermatogonial cells of mature mice [1, 6], and also in embryonic mouse tissue cells [5].

EXPERIMENTAL METHOD

Pregnant mice of the highly mutable line 101/H [11] and of the CBA line, which has a relatively low frequency of spontaneous and thiotepa-induced chromosomal lesions in bone marrow [11] and liver [4] cells, were used as the experimental model. On the 12th day of pregnancy, the rats were given an intraperitoneal injection of thiotepa in physiological saline in a dose of 2.5 mg/kg body weight According to data in the literature, the germ cell population in embryonic mouse testes on the 12th day of development consists mainly of mitotically dividing M prospermatogonia [10], which are highly sensitive to the cytotoxic action of thiotepa [7]. Control animals in each series of experiments were given an intraperitoneal injection of physiological saline.

The gametotoxic effect of antenatal exposure to thiotepa was analyzed on testes of the mature offspring of 101/H and CBA mice 3.5 months after birth. The testes of the mature animals were fixed in Bouin's solution and subjected to the usual histologic treatment. Serial sections 5 μm thick were stained with Ehrlich's hematoxylin and counterstained with eosin.

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TABLE 1. Dynamics of Spermatogenesis in 101/H and CBA Mice after Antenatal Exposure to Thiotepa in a Dose of 2.5 mg/kg at the Age of 12 Days (M ± m, n = 4-5)

Type of cells	101/H		CBA	
	control	experiment	control	experiment
Type A spermatogonia	4,1±0,7	1,7±0,3*	2,2±0,5	2,8±0,5
Spermatocytes at the preloeptotene stage	339,7±12,7	294,7±14,3*	272,8±11,4	254,3±10,8
Spermatocytes at the pachytene stage	400,1±13,7	337,8±13,4*	314,4±12,0	295,7±10,8
Spermatids at stage 7 of development	979,0±37,5	765,7±27,5*	662,1±26,8	627,7±29,2

Legend. *$p < 0.05$.

The dynamics of disturbances of spermatogenesis in the reproductive period in the first-generation offspring as a result of the damaging action of thiotepa during intrauterine development was investigated by means of quantitative karyologic analysis of the generations of germ cells at stage VII of the cycle of the spermatogenic epithelium (CSE) [9, 12].

EXPERIMENTAL RESULTS

The results of quantitative karyologic analysis of germ cell generations in the sexually mature offspring of the mice indicate the presence of significantly fewer type A spermatogonia in the seminiferous tubules of intact CBA mice at stage VIII of CSE, calculated per 100 Sertoli cells compared with the 101/H line ($p < 0.05$; Table 1). Generations of spermatocytes at meiotic stages of development (preleptotene and pachytene of prophase I) in the seminiferous tubules at stage VII of CSE also were smaller in number in CBA than in 101/H males ($p < 0.001$). The number of spermatids at stage 7 of development and at stage VII of CSE also was significantly less in intact male CBA than 101/H mice ($p < 0.001$). Thus quantitative analysis of the dynamics of spermatogenesis in intact animals revealed the existence of genotypic differences in the intensity of this process in CBA and 101/H mice.

Analysis of the late consequences of the antenatal gametotoxic effect of thiotepa also showed interlinear differences as regards the ability of the prospermatogonia to restore the germ cell pool in time for puberty. For instance, a single injection of thiotepa in a dose of 2 5 mg/kg during the period of high proliferative activity of the M prospermatogonia (12th day of antenatal development) led to a decrease in the numbers of all germ cell generations analyzed at stage VII of CSE in the seminiferous tubules of the offspring of 101/H mice compared with the control: type A spermatogonia ($p < 0.01$), spermatocytes at the preleptotene stage ($p < 0.05$) and the pachytene stage ($p < 0.01$), and also the number of spermatids at stage 7 of development ($p < 0.001$; see Table 1). The decrease in the number of spermatocytes at the preleptotene and pachytene stages and the number of spermatids in stage 7 of development in the testes of the CBA mice was not significant, and was evidently the result of the genetically determined high regenerative potential of the prospermatogonia of this line of mice, and also their greater resistance to the action of thiotepa.

In our previous investigations of the effect of thiotepa, when it was administered to pregnant animals in a dose of 5 mg/kg on the 12th day of development a significant decrease was found in the germ cell population of both male and female fetuses toward the end of the antenatal period of development [2, 4]. These findings indicate equal sensitivity of the proliferating oögonia and of the parallel (existing at the same period of antenatal development) germ cell population in the testes (M prospermatogonia) to the action of thiotepa. According to data in the literature, the high degree of sensitivity of mitotically dividing prospermatogonia also is observed to the action of other cytostatic and alkylating agents [8].

It was shown previously that embryonic mouse somatic cells can repair injuries induced in DNA by thiotepa. Under these circumstances the repair process is less efficient in 101/H than in CBA mice, suggesting the existence of positive correlation between the increase in sensitivity of 101/H mice to the mutagenic action of thiotepa and the reduced effectiveness of repair processes [5]. We know from the literature that M prospermatogonia are more sensitive to the genotoxic than to the cytotoxic action of damaging factors [3]. Interlinear differences in the level of regenerative potential of M prospermatogonia of mice of the 101/H and CBA lines, discovered in the present investigation, may evidently reflect the genetically determined different degree of efficacy of repair processes in germ cells.

The discovery of the late gametotropic effect of thiotepa in this investigation is thus evidence of the risk of development of gametopathies in the antenatal period of ontogeny.

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