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ACTION OF THIOTEPA ON THE DIFFERENTIATING OOCYTE POPULATION OF CBA, 101/H, AND AKR MICE

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UDC 615.277.3.015.44:612.622

KEY WORDS: thiotepa; oocytes.

A previous study of the action of the trifunctional alkylating agent thiotepa on mouse somatic embryonic cells showed that this mutagen, like certain other alkylating agents, induces the formation of cross-bridges in the DNA molecule, the number of which rises slowly with time [8]. This injury may be very important for determination of the cytotoxic, cytostatic, and mutagenic action of these compounds on mammlian cells. The mutagenic action of alkylating agents on somatic cell chromosomes has been studied sufficiently well [3, 8]. It has also been shown [6] that if thiotepa is given to males it induces dominant lethal mutations in mature spermatozoa and spermatids of mice which are realized in progeny obtained from males treated with this mutagen. The action of alkylating agents on differentiating female sex cells has been inadequately studied although we know that extremal influences acting on female sex cells in the period of antenatal oogenesis may be the cause of development of partial or total sterility of individuals in the postnatal period [13, 15].

This paper gives the results of a quantitative cytological assessment of the progress of oogenesis in mice after injection of thiotepa into females on the 12th day of pregnancy. According to data in the literature [9] the population of sex cells in embryonic mouse ovaries on the 12th day of development consists of proliferating oogonia and preleptotene oocytes.

EXPERIMENTAL METHOD

Pregnant CBA, 101/H, and AKR mice kept under standard conditions in the vivarium of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR, were used. The females were mated with males (two females to one male) on one night. The first day of pregnancy was taken to be the day of removal of the females from the males. On the 12th day of pregnancy the mice were given an intraperitoneal injection of thiotepa in physiological saline in a dose of 5 mg/kg body weight and in a volume of 0.5 ml/20 g body weight [2]. The animals were killed on the 19th day of pregnancy. During this period of development the majority of oocytes in the embryonic ovaries were in the pachytene stage. Fetuses at the same time of development taken from intact females, served as the control.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 6, pp. 110-113, June, 1983. Original article submitted November 18, 1982.

TABLE 1. Relative Numbers of Oocytes in Different Stages of Development in Ovaries of 19-Day Mouse Fetuses after Injection of Thiotepa into Females on 12th Day of Pregnancy

Group of animals	Line	Number of fetuses	Number of cells counted		Number of oocytes as a fraction of total number of cells counted, %	Pachytene index	Diplotene index
			total	sex cells		% of total number of oocytes counted	
Control	CBA	5	30 130	4500	14,9±0,6	89,7±2,2	9,2±1,9
	101/H	6	18 860	4513	25,6±2,3	96,6±1,3	0,7±0,7
	AKR	5	20 940	4368	21,6±1,8	98,8±0,3	0,1±0,0
Experiment	CBA	2	11 775	208	1,8±0,1	80,4±0,9	0,8±0,8
	101/H	5	58 927	1746	3,0±0,2	91,2±3,9	5,5±3,0
	AKR	6	82 705	995	1,4±0,4	95,8±1,5	2,6±0,9

After removal the fetuses were fixed in Bouin's solution. The ovaries were then removed and subjected to standard histological treatment. Paraffin sections 7 μ thick were stained with Ehrlich's hematoxylin or with hematoxylin and eosin. Oocytes in different stages of prophase of meiosis I were counted (preleptotene condensation of chromosomes, preleptotene decondensation of chromosomes, leptotene, zygotene, pachytene, and diplotene) using the scheme adopted previously [5], and their number was expressed as a percentage of the total number of oocytes counted. The number of pycnotic cells also was determined as a fraction of a total number counted. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results of determination of the number of oocytes in different stages in the ovaries of fetuses from CBA, 101/H, and AKR mice on the 19th day of development are given in Table 1. In intact CBA mice the number of oocytes was significantly lower than in 101/H and AKR mice ($P < 0.002$ and $P < 0.01$ respectively). Mice of the last two lines did not differ with respect to this parameter. Quantitative analysis of the composition of the oocyte population on the 19th day of pregnancy by stages of prophases of meiosis I enabled pachytene and diplotene indices (the number of oocytes in pachytene and, correspondingly, in diplotene as a ratio of the total number of oocytes, in per cent) to be distinguished as criteria of evaluation. In the control group of fetuses of CBA mice the pachytene index was significantly lower than in 101/H and AKR ($P < 0.01$ and $P < 0.05$ respectively). Differences in pachytene index between 101/H and AKR mice were not significant. The diplotene index, which reflects readiness of the cells to proceed (after conjugation and crossing over) into the stage of follicle formation, was higher in CBA mice than in 101/H and AKR mice. On the basis of these data it can be postulated that oogenesis in CBA mice is distinguished by further progression than in 101/H and AKR mice, which show practically no difference with respect to this index.

Injection of thiotepa into the females on the 12th day of development caused a marked decrease in the number of oocytes in 19-day fetuses.

In fetuses of CBA mice the number of oocytes in the ovary was reduced by 13%, but in 101/H and AKR mice it was reduced by 20-23% (Table 1). The pycnotic index in mice of all three lines was very small and virtually indistinguishable in the control groups (0.24-0.25%). In the experimental groups of CBA and 101/H mice a significant increase was observed in the number of pycnotic cells (0.89 and 0.85%, $P < 0.02$ and $P < 0.01$ respectively). The results in Table 1 also indicate a certain change in progression of the oocytes in the experimental animals compared with the control. A significant decrease in the pachytene and diplotene fractions and an increase in the zygotene fraction took place in the ovaries of the CBA mice. In 101/H mice a tendency was observed for the oocytes to be held up in prezygotene stages and for the number of degenerating pachytenes to increase. In AKR mice some delay in progress of the oocytes was observed in the stage of preleptotene condensation of chromosomes.

The results are evidence that quantitative analysis of the oocyte population and of the ratio between the different stages of prophase of meiosis I can shed light on the degrees of progression of oogenesis and of differences in its course both between different lines of mice and between control and experimental animals. They showed the existence of interlinear differences in size of the oocyte population and the degree of synchronization of their development in the prophase of meiosis I.

We observed that the fraction of oocytes in CBA fetuses was appreciably smaller than in 101/H and AKR mice. In CBA mice oocytes enter the diplotene stage earlier than in 101/H and AKR mice relative to the times of intrauterine development. This finding agrees with the results of investigations which showed the greater progress of oogenesis in newborn CBA mice than in mice of line A [10].

The present experiments showed that thiotepa, given as a single dose at a time when the population of sex cells consists of oogonia and preleptotene oocytes, causes a marked decrease in the number of oocytes toward the end of the antenatal period (Table 1).

It was shown previously that interlinear differences exist in the sensitivity of sex cells at definite stages of their development to harmful agencies [12]. In the present experiments female sex cells in mice of lines 101/H and AKR were more sensitive to thiotepa than in CBA mice, for an increase in their fraction by 20-23% was observed (Table 1), compared with only 13% in CBA mice.

The great resistance of CBA mice to the mutagenic action of thiotepa has frequently been mentioned in the literature. This is associated with the greater potential for intracellular repair of DNA in the CBA line [4, 7, 8]. Differential analysis of the ratio between stages of prophase of meiosis I, using pachytene and diplotene indices in mice of different lines after treatment with thiotepa leads to the conclusion that interlinear differences exist in the response to the action of this compound, as shown not only by a decrease in the total number of oocytes but also a change in the degree of progression of oogenesis. In CBA mice a tendency is observed for delay of the progressive development of oocytes: a decrease in the number of pachytenes and diplotenes on account of an increase in the number of prepachytene stages (compared with the control). In mice of the 101/H line a tendency was found for the oocytes to be held up in prezygotene stages. In AKR mice a significant increase was found in the number of oocytes in diplotene.

A considerable decrease in the number of oocytes with the develpment of ovarian dysgenesis has been observed in rats and mice treated antenatally with stathmokinetic or radioactive substances [13, 15]. The answer to the question of the pathogenesis of dysgenesis and the precise mechanisms involved in the initial stages of interaction between the harmful agent and the sex cells has not yet been obtained.

What could be the cause of the reduction in the number of sex cells under the influence of thiotepa in our experiments? Thiotepa interacts with the DNA molecule. It was shown previously that this mutagen causes the formation of cross-linkages in the DNA molecule between strands and within strands, and this may be an obstacle to further replication, and may perhaps also involve processes of cell differentiation. In our opinion, among the oogonia those most sensitive to thiotepa may be cells in the S period and in mitosis. Evidently injuries to DNA on the one hand prevent further proliferation of oogonia, and on the other hand they perhaps involve certain stages on the pathway of differentiation of the oogonium into a meiocyte. There is evidence that premeiotic DNA synthesis takes place in sex cells in the initial stages of prophase of meiosis I [11, 14]. It can accordingly be postulated that thiotepa, by interacting with DNA, also damages oocytes in the early (preleptotene?) stages of prophase of meiosis I.

Indirect pathways of injury by this compound, i.e., those not directly damaging the sex cells, likewise cannot be ruled out. The gametotoxic action of thiotepa, leading to death of oocytes in insects, was exerted through inhibition of endomitosis in nurse cells [1].

A sharp decrease, but not complete disappearance, of the oocyte population before birth (the day of sacrifice) after injection of thiotepa on the 12th day of pregnancy suggests that a certain proportion of the sex cells was sensitive to this compound at the time of treatment: this was either the progeny of oogonia which remained intact after treatment with thiotepa or oocytes in the preleptotene stage, but possibly in that subphase of it which was already shown to be resistant to thiotepa, and which continued the progression of meiosis after inactivation of the compound in the body.

The overwhelming majority of oocytes (80-99%) in fetuses on the 19th day of pregnancy in both the control and the experimental animals (i.e., those surviving after treatment with thiotepa) reached the pachytene stage. This suggests that general progression of oocytes in prophase of meiosis I (i.e., the quantitative ratio between oocytes in particular stages) does not necessarily depend on the total size of the sex cell population and is controlled by different mechanisms.

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CHANGES IN EPIDERMAL G_2-CHALONE CONTENT AND MITOTIC ACTIVITY OF VAGINAL EPITHELIAL CELLS OF OVARIECTOMIZED RATS AFTER STIMULATION OF PROLIFERATION BY ESTRADIOL

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UDC 618.11-089.87-092.9-07:618.15-018.15+618.15-018.73:576.353.7]-02:615.256.51

KEY WORDS: estradiol; G_2 chalone; mitotic activity.

According to Khlopin's histogenetic classification the vaginal epithelium is one of the epidermal tissues. Accordingly the proliferation of the cells of this epithelium may evidently be regulated by epidermal chalones. For instance, their inhibitory action on DNA synthesis and mitosis of epitheliocytes has been demonstrated [4], and the presence of an endogenous epidermal G_2 chalone in this epithelium has been found immunologically [5, 7]. The vaginal epithelium is a hormone-dependent tissue. Ovariectomy leads to its hypoplasia, and administration of estrogens against this background is a powerful stimulus to proliferation, causing a marked increase in the index of labeled nuclei (ILN) and mitotic coefficient (MC) of the cells [6, 9, 10]. To judge from the few available data, the increase in mitotic activity after such stimulation is preceded by a fall in the G_2 chalone level in the epithelium [1, 8]. However, in the investigations cited G_2 chalone was determined in extracts made from the whole organ, by Mancini's immunodiffusion method, and for that reason its concentration may have depended on the completeness of extraction and on various other causes.

For the reasons mentioned above, in the investigation now described an attempt was made to characterize changes in the epidermal G_2 chalone content and to compare them with data on mitotic activity in the vaginal epithelium of ovariectomized rats at different times after stimulation of proliferation by estradiol, using a technique of quantitative immunomorphology, giving a more adequate estimate of its concentration actually in the epithelial cells.

Laboratory of Experimental Histology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 6, pp. 113-115, June, 1983. Original article submitted August 13, 1982.

 0007-4888/83/9506-0864$07.50