EFFECT OF MATERNAL GENOTYPE ON THE RATE OF DOMINANT LETHAL MUTATIONS INDUCED BY THIOPHOSPHAMIDE IN MATURE MOUSE SPERMATOZOA

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Male CBA mice were treated with thiophosphamide (6 mg/kg) and crossed with A/He, C57BL/6, C57BL/Mib and fidget females. The minimal dominant lethal mutation rate was recorded in the progeny of the C57BL/Mib females. It is suggested that the most active repair of premutation injuries takes place in the ova of females of this line.

KEY WORDS: dominant lethal mutations; repair; premutation injuries.

Certain types of chromosomal aberrations induced by mutagens in sex cells are one of the chief causes of death of developing embryos, i.e., they are manifested as dominant lethal mutations (DLM). When mature spermatozoa are treated with mutagens, primary injuries to the chromosomes are not realized as chromosomal mutations until fertilization and the first cleavage divisions. Repair processes can be classed among processes which influence realization of primary injuries to DNA and chromosomes. By contrast with male sex cells, in which repair has been observed in the early stages of development but is absent in mature spermatozoa [8, 9, 12], repair in ova takes place at all stages of development [5].

Probably the ovum can repair not only its own DNA, but also DNA of the spermatozoon treated with mutagen before fertilization. This hypothesis has been confirmed by some indirect data [2, 6, 7, 10]. A genetic analysis of factors controlling maternal repair has recently been undertaken in lines of Drosophila defective for this process [6]. So far very few such investigations have been carried out on mice. Some workers, having crossed mice, have assessed the rate of DLM induced in spermatids [2], rather than in mature spermatozoa. However, partial repair of DNA injuries can take place in spermatids [8], and this makes interpretation of the results of such experiments difficult.

The object of this investigation was to assess the repair activity of the ova of mice of different lines. Frequencies of DLM induced by thiophosphamide during crossing of male mice of the tester line treated with this compound and intact females served as an evaluation criterion. Only those DLM induced in mature spermatozoa were recorded. By carrying out experiments under these conditions the influence of repair processes taking place in the male sex cells is reduced to a minimum.

EXPERIMENTAL METHOD

Mice aged 2-3 months were used as the experimental animals: CBA males, supplied by the "stolbovaya" nursery, and sexually mature virgin females of the C57BL/6 ("Stolbovaya" nursery), A/He, C57BL/Mib, and fidget lines, reared in the animal house of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. The fidget mice were obtained from Jackson's laboratory, where they were crossed 5 times in succession with the inbred line BALB/c, and in the USSR they were crossed many times in succession with C57BL/Mib mice, as a result of which they became black in color and acquired pigmented eyes. In mice homozygous for the fidget mutation, developmental defects of the eye, inner ear, cerebellar cortex, etc., were observed [1].

Thiophosphamide, in a dose of 6 mg/kg (this dose was chosen after preliminary experiments using a series of doses), was injected intraperitoneally into the males in Hanks' solution, after which each male was mated for 1 week with two females of one or other line. The females were examined 10-12 days after removal

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TABLE 1. Number of Implantation Sites and Living Embryos of Female Mice of Different Lines when Crossed with CBA Males Treated with Thiosphosphamide

Line of females	Group	n	m	N	$\bar{m} \pm S_m$	$\bar{N} \pm S_N$	G_{total}	$G_{p.i}$
A/He	Control	39	366	315	9,38±0,32	8,07±0,30	0,60	0,39
	Experiment	26	161	85	6,23±0,44	3,27±0,40		
C57BL/6	Control	34	261	232	7,67±0,29	6,82±0,28	0,61	0,44
	Experiment	31	168	84	5,42±0,45	2,70±0,33		
C57BL/Mib	Control	24	199	180	8,29±0,29	7,41±0,31	0,44	0,27
	Experiment	29	185	123	6,37±0,49	4,42±0,46		
Fidget	Control	14	102	90	7,28±0,44	6,42±0,41	0,59	0,36
	Experiment	9	42	24	4,67±0,52	2,67±0,31		

from the males. The number of implantation sites and the number of dying and living embryos were determined. Corpora lutea of pregnancy were not counted because visual determination of this parameter in mice is insufficiently accurate [3, 4].

The frequency of induced DLM was estimated as the total induced embryonic mortality (I_{total}) and the induced postimplantation mortality of the embryos ($I_{p.i.}$). These indices were calculated by the following equations [11]:

$$I_{total} = \left|1 - \frac{N_e/n_e}{N_c/n_c}\right|; \quad I_{p.i} = 1 - \frac{N_e/m_e}{N_c/m_c},$$

where N_e and N_c are the number of living embryos; m_e and m_c the number of implantation sites, and n_e and n_c the number of pregnant females in the experimental and control groups respectively.

EXPERIMENTAL RESULTS

Data on the number of implantation sites and living embryos of females of different lines after mating with CBA males, treated or untreated with thiophosphamide, are given in Table 1. Results of calculation of the induced total and postimplantation mortality of the embryos are also given in the same table.

It will be clear from Table 1 that the induced total embryonic mortality was practically the same in females of A/He and C57BL/6 lines. The females of these lines differed only in the induced postimplantation mortality of the embryos. The level of induced total and postimplantation embryonic mortality of C57BL/Mib mice was significantly lower than in mice of the first two lines.

It was also interesting to examine the results obtained with mutant fidget mice, although, because of the small number of these animals, they can only be regarded as preliminary.

As Table 1 shows, the induced total embryonic mortality after crossing CBA males treated with thiophosphamide and fidget females was higher than when the same males were crossed with C57BL/Mib females, namely 0.59, i.e., close to the level of mortality in A/He and C57BL/6 mice. This suggests that the fidget mutation, relative to which the mice of this line are known to differ from C57BL/Mib mice, affects not only the formation of developmental defects [1], but also induced embryonic mortality.

Differences in the frequency of DLM induced by thiophosphamide in mature spermatozoa of CBA males, observed after crossing with females of different lines, could indicate that injuries to chromosomes of spermatozoa are realized as aberrations with different frequency depending on the genotypic features of the ova in the females. Assuming that the realization of premutation injuries of spermatozoa as chromosomal mutations is influenced by DNA and chromosome repair processes taking place in the mouse ovum, it can be concluded from the results now obtained that among the lines of mice studied those of line C57BL/Mib are distinguished by the highest activity of these processes.

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PROTEINS OF THE RETINA AND ITS PIGMENTED EPITHELIUM IN HEREDITARY DEGENERATION OF THE RETINA

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The assortments of water-soluble and membrane proteins of the retina and its pigmented epithelium in Campbell (albino) rats with hereditary degeneration of the retina and in healthy Wistar rats were studied by electrophoresis in polyacrylamide gel. Early changes were shown to be detectable in the assortment of retinal proteins of the diseased animals; the first proteins to undergo changes were found to be neither cyclic nucleotide phosphodiesterase nor opsin. Changes in the set of proteins of the pigmented epithelium were observed much later.

KEY WORDS: retinal protein; proteins of the pigmented epithelium; hereditary degeneration of the retina.

The location of the primary lesion in the eye – whether in the retina or pigmented epithelium (PE) – in hereditary degeneration of the retina is a problem that is still unsolved [4, 10, 12].

It was accordingly decided to compare changes in the protein composition of the retina and the pigmented epithelium in relatively early stages of the disease. In the present investigation the assortments of water-soluble and membrane proteins of the retina and PE in Campbell rats with hereditary degeneration of the retina and in healthy Wistar rats at different periods of postnatal life were studied by electrophoresis.

EXPERIMENTAL METHOD

Electrophoresis of the proteins in acrylamide gel was carried out by the methods in [1, 6, 11], using the supernatant as the fraction of water-soluble proteins and the residue obtained after centrifugation of a homogenate of the retina or PE at 13,000g for 15 min as membrane proteins. Cyclic GMP phosphodiesterase (PDE) in the gels after electrophoresis was identified by the method in [7]. Opsin was identified by electrophoresis of the membrane proteins of the outer segments of the rods (OSR) of a rat, which were isolated in the same way as bovine OSR [2]. Protein was determined by Lowry's method [9]; protein in the residue was calculated as the difference between protein of the homogenate and of the supernatant.

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