PREIMPLANTATION EMBRYONIC MORTALITY AS A METHOD OF STUDYING STRAIN DIFFERENCES IN THE REPARATIVE ACTIVITY OF MOUSE OOCYTES

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The ability of chromosomal disturbances induced in the sex cells of mice to undergo repair has been demonstrated in experiments on irradiated animals [1]. The present writers showed previously that activity aimed at repair of chromosomal injuries in male gametes can be compared by the dominant lethal mutations technique in oocytes of different strains of female mice [2].

The object of this investigation was to verify this conclusion by cytologic analysis of the preimplantation mortality.

EXPERIMENTAL METHOD

Female mice of three strains (101/HG, C57BL/6G, and CBA/LacG) and male (BALB/cG × DBA/2G)F_1 hybrids were used. The age of the animals was 2.5-3.5 months. A solution of thioTEPA in a dose of 2 mg/kg body weight was injected into the male mice intraperitoneally. One week later, each male was mated overnight with three virgin females (one of each strain), which were removed in the morning and tested for the presence of a vaginal plug as evidence of mating. Intact males (control) were kept under similar conditions. The effect of the mutagen was determined from the embryonic mortality in females fertilized during the second week (action on late spermatids) after injection, on the basis of changes in embryonic mortality on the 4th day of development. Cytologic analysis of the embryos was carried out on preparations obtained by Tarkowski's method in Dyban's modification [3]. The preparations were stained with lactic acid–orcein and the number of blastomere nuclei was counted. The effect of the mutagen was determined from the increase in embryonic mortality before implantation, detected by the cessation of cleavage of the embryos at the 2-20 blastomeres stage.

EXPERIMENTAL RESULTS

Comparison of the spontaneous embryonic mortality in mice of different strains showed (Table 1) that spontaneous preimplantation embryonic mortality was highest in CBA/LacG females. However, calculation of the mean number of cells per embryo in females of the genotypes tested revealed a completely different picture in the control, as follows: Mortality was lowest in CBA/LacG females (29.5), highest in C57BL/6G (42.1), and intermediate in strain 101/HG (33.5). There is thus reason to suppose that implantation in strain CBA/LacG is shifted to a relatively later time. Naturally this hypothesis requires special investigation. The level of spontaneous embryonic mortality before implantation was probably high in CBA/LacG females because of inclusion of embryos (retarded in development) capable of further development, among the number of arrested embryos. As the results show, after the action of thioTEPA on late spermatids the embryonic mortality in the preimplantation period in all experimental groups was statistically significantly ($P < 0.05$ and < 0.001) higher than the control. Differences also were found between the experimental groups of females. The maximal value of the preimplantation embryonic mortality was recorded in 101/HG females, minimal in CBA/LacG females, and an intermediate value was found in C57BL/6G females. Differences between strains 101/HG and C57BL/6G did not reach the necessary level of significance. As regards differences between the groups of 101/HG and CBA/LacG females, these were highly significant ($P < 0.001$). The significance of differences in the level of preimplantation

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TABLE 1. Results of Investigation of Preimplantation Embryonic Mortality in Females of Different Genotypes

Group of animals	Strain of female mice	Number of females	Number of embryos and oocytes flushed out					
			total	including dead embryos		confidence interval	undeveloped oocytes	
				absolute	%		absolute	%
Experimental	101/HG	32	260	99	38,8	33,0—44,7	18	6,8
	C57BL/6G	30	246	77	31,3	25,6—37,2	29	11,8
	CBA/LacG	28	242	53	21,9	16,9—27,3	13	5,4
Control	101/HG	35	281	22	7,8	5,0—11,2	20	7,2
	C57BL/6G	28	228	10	4,4	2,1—7,4	28	12,4
	CBA/LacG	23	200	24	12,0	7,8—16,8	12	6,0

Legend. Confidence interval calculated at P = 0.95.

embryonic mortality between C57BL/6G and CBA/LacG females corresponded to the first threshold of significance ($P < 0.05$).

The same injuries, induced by thioTEPA in late spermatids of F_1CD males, thus evoke quantitatively different degrees of preimplantation embryonic mortality in females of different genotypes. Consequently, this suggests that fertilized oocytes have the ability to repair some of the injuries carried by the paternal genome. To the greatest degree among the genotypes studied this property was manifested in CBA/LacG females. It was not an aim of the present investigation to estimate the frequency of chromosomal abberations, but previous investigations using cytogenetic analysis revealed that the dying embryos had chromosomal aberrations [4, 5].

Calculations showed that any visible chromosomal aberrations are lethal for their carriers [6]. Similar results were obtained in experiments with triethylenemelamine [7-9]. Hence all or nearly all dominant lethals causing death of the embryo in the initial stages of development are chromosomal aberrations leading to loss of genetic material. Realization of the maximal effect of a chemical mutagen requires DNA replication [10]. The degree of realization of chromosomal aberrations and their number reflect the functioning of repair systems. The results of the present investigation are evidence that repair activity in oocytes of CBA/LacG females is about 18% greater than in 101/HG females, and 9% greater than in C57BL/6G females.

Strain differences thus exist in ability to repair chromosomal injuries induced by thioTEPA.

The results of these investigations show that the preimplantation embryonic mortality can be used as a method of studying strain differences in reparative activity of mouse oocytes. Meanwhile, if a chemical compound is being tested for mutagenicity by the dominant lethal mutation method it must be recalled that the effect of its action depends not only on the dose and the degree of activity of the mutagen, but also on activity of function of the repair system of the oocyte.

LITERATURE CITED

1. W. L. Russell, in: Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, F. H. Sobels, ed., Oxford (1963), pp. 205-218.
2. A. M. Malashenko and N. I. Surkova, Genetika, No. 2, 105 (1975).
3. A. P. Dyban and L. D. Udalova, Genetika, No. 4, 52 (1976).
4. A. M. Malashenko, Kh. Kh. Semenov, G. P. Selezneva, et al., Genetika, No. 1, 52 (1978).
5. Kh. Kh. Semenov and A. M. Malashenko, Byull. Éksp. Biol. Med., No. 10, 107 (1975).
6. J. G. Brewen, H. S. Payne, K. P. Jones, et al., Mutat. Res., 33, 239 (1975).
7. K. Burki and W. Sheridan, Mutat. Res., 49, 259 (1971).
8. B. E. Matter and I. Jaeger, Mutat. Res., 33, 251 (1975).
9. K. Burki and W. Sheridan, Mutat. Res., 52, 107 (1978).
10. J. G. Brewen and H. S. Payne, Mutat. Res., 50, 85 (1978).