# TERATOGENIC ACTION OF THIOPHOSPHAMIDE ON MICE OF DIFFERENT GENOTYPES

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Genotype is known to have an important effect on sensitivity of organisms to the mutagenic action of chemical and physical factors. It was shown previously [2] that the level of chromosomal aberrations induced by thiophosphamide (thiotepa) in somatic cells of 101/H mice is higher than in CBA mice. It was accordingly interesting to compare the teratogenic effect of thiotepa in mice of these lines. The basic assumption was that one cause of disturbance of normal development in the case of exposure of embryos to alkylating agents or ionizing radiations may be mass cell death induced by these factors [1, 4]. In particular, cells with gross structural chromosomal aberrations die as a rule in the first division cycle after treatment. Cells with nonlethal structural changes — with certain minor deletions, inversions, reciprocal translocations — can disturb normal tissue differentiation. As a result the structure and function of organs with mosaic cell composition may be abnormal.

In the investigation described below the teratogenic action of thiotepa was studied in experiments on 101/H and CBA mice, which differ in their response to mutagenic action.

## EXPERIMENTAL METHOD

Inbred mice of lines 101/H and CBA were kept under standard conditions in the animal house of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. Thiotepa in physiological saline was injected intraperitoneally in a dose of 5 mg/kg body weight into females on the 12th day of pregnancy (the period of active organogenesis). The dose of the compound was chosen in preliminary experiments with a view to minimizing the embryolethal effect. Some females were killed 11-12 h after injection of the compound to determine the levels of aberrant metaphases in the embryonic liver [3]. The rest of the female mice were killed on the 19th day of pregnancy; fetuses were removed from the uterine cornua and gently dried on filter paper, weighed, fixed with 96% ethanol, and stained by Dawson's method to reveal zones of ossification. The crown-rump length of the stained fetuses was measured under the MBS-2 microscope (4.8×) with an ocular micrometer. The measurements were expressed in ocular micrometer units. The number of centers of ossification in the sternum and the number of pairs of ribs were determined in the fetuses, and developmental anomalies of the axial skeleton, limbs, and skull were described. Parallel investigations were carried out on control fetuses from intact mothers.

## EXPERIMENTAL RESULTS

The action of thiotepa on developing mouse embryos was manifested as a decrease in weight of the fetuses of the experimental groups of animals compared with the control (Table 1 ). The mean weights of fetuses from intact 101/H and CBA mice differed significantly ($P < 0.001$). Differences in weight of the fetuses of the experimental groups compared with the control also were highly significant ($P < 0.001$ for both lines). The mean weight of the fetuses of mice of the experimental group of the 101/H line was 55% of the control, compared with 64% for CBA.

The results of measurement of the crown-rump length of the fetuses are given in Table 2. The mean lengths of the fetuses from intact 101/H and CBA (control group) fetuses differed significantly, as also did the mean lengths of the experimental and control fetuses ($P < 0.001$ for both lines). The mean length of the fetuses of the experimental (101/H) group of mice was 64% of the control, compared with 78% for CBA.

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TABLE 1. Effect of Thiotepa on Weight of Mouse Fetuses (in g) on 19th Day of Development

| Statistical parameter | Control | | Experiment | |
|---|---|---|---|---|
| | 101/H | CBA | 101/H | CBA |
| $n$ | 179 | 318 | 147 | 162 |
| $\bar{x}$ | 1,1051 | 0,9297 | 0,6121 | 0,6112 |
| $S_{\bar{x}}$ | 0,0179 | 0,0062 | 0,0130 | 0,0061 |
| $\sigma^2$ | 0,0577 | 0,0121 | 0,0248 | 0,0061 |
| $V$, % | 21,73 | 11,84 | 25,74 | 12,79 |
| $S_V$ | 1,15 | 0,47 | 1,50 | 0,71 |

TABLE 2. Effect of Thiotepa on Length of Mouse Fetuses on 19th Day of Development

| Statistical parameter | Control | | Experiment | |
|---|---|---|---|---|
| | 101/H | CBA | 101/H | CBA |
| $n$ | 62 | 216 | 104 | 152 |
| $\bar{X}$ | 13,06 | 11,95 | 8,37 | 9,36 |
| $S_{\bar{x}}$ | 0,21 | 0,05 | 0,16 | 0,06 |
| $\sigma^2$ | 2,87 | 0,56 | 2,77 | 0,50 |
| $V$, % | 12,97 | 6,26 | 19,91 | 7,55 |
| $S_V$, % | 1,16 | 0,30 | 1,38 | 0,43 |

TABLE 3. Number of 19-Day Fetuses with no Anomalies of the Locomotor System after Treatment with Thiotepa (in percent)

| Statistical parameter | Control | | Experiment | |
|---|---|---|---|---|
| | 101/H | CBA | 101/H | CBA |
| $n$ | 155 | 284 | 122 | 157 |
| $P$, % | 93,6 | 96,5 | 4,9 | 38,8 |
| $S_p$, % | 2,0 | 1,1 | 1,9 | 3,9 |

Injection of thiotepa into pregnant female 101/H and CBA mice thus leads to a decrease in weight and length of the 19-day fetuses. The decrease was more marked in 101/H than in CBA mice. The state of the locomotor system of the fetuses was used as principal parameter of the teratogenic action of thiotepa. The compound was found to disturb development of the skull, ribs, and long and pelvic bones. In the severest cases there was a decrease in the number of ribs or even their total absence, accompanied by absence of centers of ossification in the sternum, absence of the fibula, deformation of the remaining limb bones and of the caudal region of the spine, and also marked underdevelopment of the cranial bones.

To compare the teratogenic action of thiotepa on the mouse embryos quantitatively the number of fetuses with and without anomalies of the locomotor system was counted. Table 3 gives the number of fetuses without deviations from the standard (norm) in 101/H and CBA mice in the experimental and control groups. As Table 3 shows, the teratogenic action of thiotepa was much more marked in 101/H than in CBA mice (5 and 39% of fetuses, respectively, had no anomalies).

When the percentage of cells with structural chromosomal aberrations in the embryonic mouse liver was determined after the same dose of thiotepa, the following results were obtained: 76 ± 5.1% of aberrant cells in 101/H mice and 55.6 ± 3.2% in CBA mice.

The results are evidence of correlation between sensitivity of mice of the two lines studied to the mutagenic and teratogenic action of the alkylating agent thiotepa and they show that this sensitivity is largely determined by the recipient's genotype.

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# EFFECT OF TRANSPOSONS Tn1 AND Tn9 ON GENETIC CONTROL SYSTEM OF TRANSFER AND INCOMPATIBILITY FUNCTIONS OF pAP19-1 Col-PLASMID

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The writers previously showed that derepressed (drd) variants of the F-like Ent[1]-plasmid pAP10-2 can be obtained by incorporating transposon Tn9 into its structure [2]. The object of this investigation was to induce drd-mutants of F-like Col-plasmid pAP19-1 with the aid of transposons Tn1 and Tn9 and also to study regulation of transfer (Tra) function and compatibility with F-like plasmids of different incompatibility groups in these mutants.

## EXPERIMENTAL METHOD

Standard strains of *Escherichia coli* K-12 with chromosomal genes of resistance to streptomycin (C600, AP106) or to nalidixic acid (AP115, AP132) were used.

Genetic markers of the test plasmids (resistance to antibiotics, transmissibility, sensitivity to donor-specific phages), and conjugation transmission of plasmids and their ability to mutually inhibit transfer functions were undertaken by standard methods [3, 5]. Transposons Tn1 and Tn9 were incorporated into the structure of the test plasmid by schemes worked out previously [1, 4]. Compatibility (incompatibility) of the plasmids was determined by the standard method [7]. The plasmids and their genetic markers were named in accordance with the recommendation of Novick et al. [9].

## EXPERIMENTAL RESULTS

Plasmid pAP19-1, discovered previously [6] in cells of conditionally pathogenic strain *E. coli* AP53 (serogroup 0141) was transmitted into cells of strains *E. coli* K-12. After incorporation of transposon Tn1 or Tn9 into the structure of plasmid pAP19-1, ten drd-mutants of this plasmid containing one of the above-mentioned transposons were selected. All mutants obtained were able to make the cells containing them sensitive to donor-specific phage MS2 and highly efficient in conjugation transmission of plasmid markers. They were also sensitive to the inhibitory action of plasmid R1, with repressed Tra functions. Typical clones of bacteria with plasmid mutants, carrying transposons Tn1 and Tn9, and designated pAP19-2 and pAP19-3 (respectively), were selected for further experiments.

The results of the study of systems controlling Tra functions of these plasmids, given in Table 1, show that mutant plasmids pAP19-2 and pAP19-3 (like the original plasmid pAP19-1 and its Tn9-marked variant of repressed type, pAP19-4), are unable to inhibit transfer functions of derepressed reference plasmid Flac. Meanwhile Tra functions of drd plasmids pAP19-2 and pAP19-3 are inhibited by reference plasmids R1 and R100, which as we know are carriers of

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