ASSESSMENT OF THE CONTRIBUTION OF THIOPHOSPHAMIDE-INDUCED CHROMOSOMAL ABERRATIONS TO PREIMPLANTATION EMBRYONIC MORTALITY IN MICE

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UDC 618.33-036.88-02

A cytological analysis was made of thio-TEPA-induced preimplantation embryonic mortality in mice and the contribution of induced chromosomal aberrations was examined. Under the influence of thio-TEPA in a dose of 1.25 mg/kg cleavage was arrested in late spermatids in 27% of cases at the 2-16-cell stage and in 11.9% at the 17-22-cell stage, making a total of 38.9% compared with 6% in the control. Cytogenetic analysis of embryos consisting of 2-16 cells revealed gross structural chromosomal aberrations in 75% of metaphases suitable for analysis.

KEY WORDS: chromosomal aberrations; embryonic mortality; blastomere.

It has been shown experimentally that the dominant lethal mutations method in mice is more sensitive than cytogenetic analysis of bone marrow [2]. It is therefore widely used in the experimental study of the genetic effects of chemical compounds. Embryonic mortality in mice is exposed to some degree to the influence of uncontrollable factors, whereas chromosomal aberrations are irrefutable proof of the genetic activity of a chemical compound tested. Consequently, to make absolutely certain of the mutagenicity of a chemical compound, it is desirable not only to determine the quantitative embryonic mortality, but also to assess the contribution of chromosomal disturbances. Genetic causes of embryonic death are known to vary from gene mutations to loss of entire chromosomes.

The object of this investigation was to assess the contribution of chromosomal aberrations induced by thiophosphamide (thio-TEPA) to early embryonic mortality.

EXPERIMENTAL METHOD

To induce dominant lethal mutations, thio-TEPA was dissolved in physiological saline and injected intraperitoneally into male C57BL/6Y mice in a dose of 1.25 mg/kg. During the 7 nights following the second week of incubation, 3 to 4 virgin females were introduced into the cage of each male (BALB/c × DBA/2) F_1. The age of the animals used in the experiment did not exceed 2-3.5 months. Fertilized females were identified by the presence of a vaginal plug. The embryos were studied on the 4th day of development, when they were at the blastocyst stage and lay freely in the uterine cavity. To obtain embryos the uterine cornua were flushed out with warm medium No. 199. Females in whom the number of flushed out embryos and ova agreed exactly with the number of corpora lutea in the ovary were chosen for analysis. Chromosomal preparations were made by Tarkowsky's method [6] in Dyban's [1] modification from embryos which still remained at the 2-22-blastomere stage. The preparations were stained with aceto-orcein and metaphase plates were analyzed.

EXPERIMENTAL RESULTS

The results of these experiments show that thio-TEPA in a dose of 1.25 mg/kg, just as in a higher dose (5 mg/kg [4] also), did not impair normal fertilization. For instance, equal numbers of undeveloped ova were obtained in the experimental and control series. Their proportion in the control was 6% and in the experiment 5.4%. Doses of 1.25 and 5 mg/kg differed significantly in their action on late spermatids. For instance, whereas a dose of 5 mg/kg caused so many chromosomal injuries in the late spermatids that all the embryos died before implantation, their development arrested at 2-16-cell stages, when a dose of 1.25 mg/kg was used the proportion of embryos which remained at these stages was only 27%. The effect of dose is illustrated more convincingly still by comparison of the number of embryos remaining at the 2-8-cell stages of development.

Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 353-355, September, 1979. Original article submitted May 31, 1978.

 0007-4888/79/8809-1074$07.50

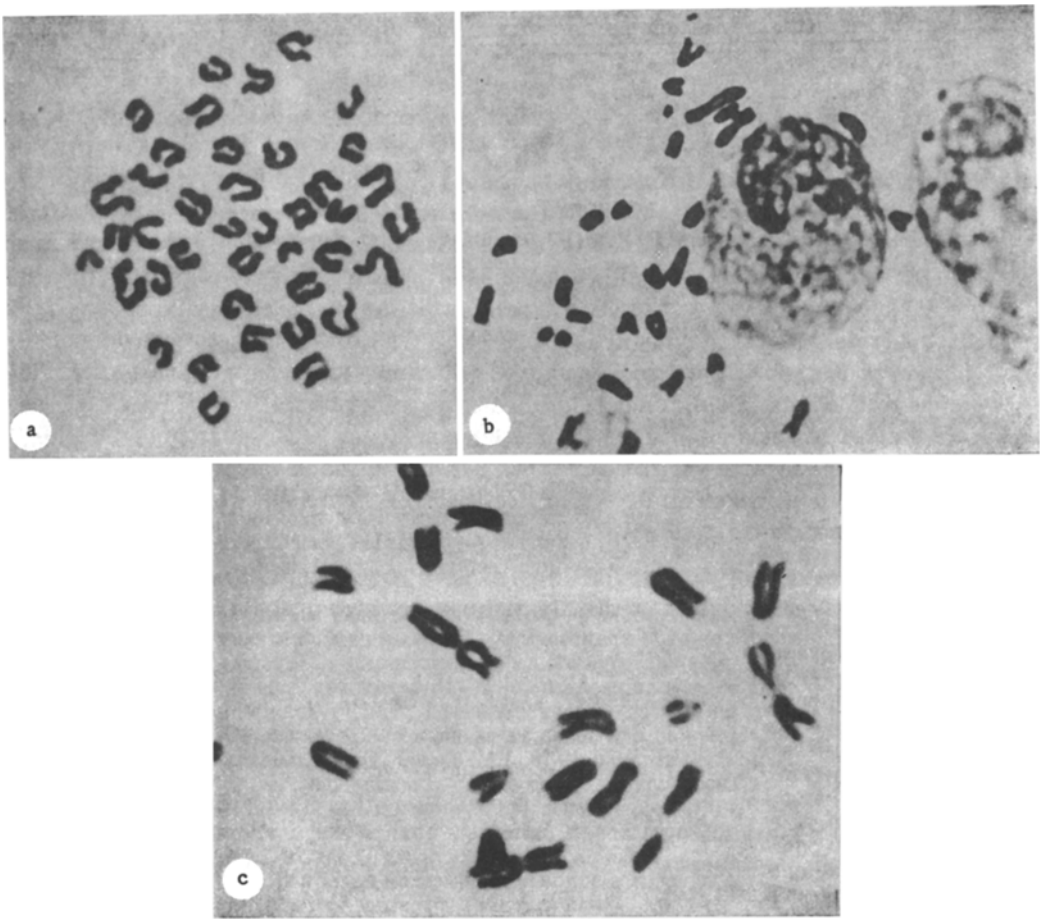

Fig. 1. Metaphase plates obtained from 3.5-day embryos. a) Normal cell (control group); b) enlarged chromosome and paired fragment; c) metacentric chromosomes. Objective 40, ocular 25 (10× 2.5).

For instance, in the experiment described above (1.25 mg/kg) they accounted for only 4.3%, whereas after the higher dose (5 mg/kg) of thio-TEPA they accounted for over half the total number of embryos (67.2%). Probably the more injuries were induced in the male sex cell, the earlier the stages at which the embryo died; accordingly, the number of blastomeres of the residual embryos may reflect the degree of damage to the genetic material.

Nevertheless, a dose of thio-TEPA of 1.25 mg/kg was highly effective, for compared with the control in which the number of embryos with arrested or retarded development did not exceed 6% (1.6% at the 2-16-blastomere and 4.4% at the 17-22-blastomere stage), the proportion of dying embryos in the experimental group was significantly greater. The number of dying embryos was 38.9%, of which 11.9% consisted of embryos at the 17-22-cell stage of development.

The fraction of normally developing embryos at the blastocyst stage in the present experiment was thus 55.7% compared with 88% in the control.

Just as in previous investigations [4], special attention was directed to comparing the structure and karyotype of the same embryos. The embryos were therefore analyzed under the microscope, and later specimens were prepared from them so that the number of blastomeres could be counted and the chromosomes in the metaphase plates investigated. It was hoped to determine to what extent chromosomal aberrations affected the rate of cleavage of the embryos. Altogether 50 embryos consisting of two to 16 blastomeres were subjected to cytogenetic analysis. Metaphases suitable for detection of chromosomal aberrations were obtained from only 24 of them. Of the 24 embryos investigated cytogenetically, 18 (75%) contained structural aberrations: fragments (in most cases, paired) and chromosomes with their structure altered as a result of translocations (abnormally long, metacentric chromosomes and one circular chromosome (Fig. 1). Similar results were obtained during a study of the effect of triethylenemelamine [5]: exchanges, fragments, micro-

nuclei, and premature condensation of chromosomes were observed in 4-8-cell embryos. The authors cited concluded that induced death of the embryos was due to losses of genetic material. Embryos consisting of 17-22 cells also were investigated cytogenetically. Among 20 metaphases suitable for analysis, structural chromosomal aberrations were found in only three. Only structural chromosomal aberrations were considered, for aneuploidy in specimens prepared by this method could be an artefact, and this had always to be taken into account. Naturally, a definite proportion of aneuploidy is the result of elimination of unstable types of aberrations (acentric fragments and rings), which are eliminated in 70% of cases after the first division, for they do not diverge toward the poles [3]. Specific difficulties in the investigation of early embryogenesis – absence of mitoses in some embryos and also despite the use of colchicine, weak spiralization and close approximation of chromatids in the cleaving embryos (especially at the 2-4-cell stages) – were reflected in the course of cytogenetic analysis. These factors probably explain the difference between the number of embryos with chromosomal aberrations and the number of embryos with arrested or retarded development. Of course the contribution of unmonitored factors in embryonic mortality cannot be ignored. In the control the total number of embryos at the 2-16-blastomere stage (5 or 1.6%) and at the 17-22-blastomere stage (14 or 4.4%) was only 19, or 6%. Three metaphases were obtained from them, but no chromosomal aberrations could be found in them. In additional prolonged control cytogenetic studies of 88 metaphases from 2.5-day embryos no structural chromosomal aberrations likewise were found.

In conjunction with the results of previous studies, those obtained in the present investigation thus indicate that gross chromosomal aberrations in mammals disturb cleavage of the ova in the initial stages (2-16 blastomeres).

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