LITERATURE CITED

1. N. M. Mironov, V. V. Lobanenkov, and V. S. Shapot, Dokl. Akad. Nauk SSSR, 254, 1269 (1980).
2. N. M. Mironov, Biokhimiya, No. 3, 503 (1987).
3. R. Berezney and D. S. Coffey, Science, 189, 291 (1975).
4. P. Chambon, Cold Spring Harbor Symp. Quant. Biol., 42, 1209 (1978).
5. C. Cremisi, Microbiol. Rev., 43, 297 (1979).
6. P. L. Jack and P. Brookes, Nucl. Acids Res., 9, 5533 (1981).
7. V. V. Lobanenkov, M. Plumb, G. H. Goodwin, and P. L. Grover, Carcinogenesis, 7, 1689 (1986).
8. T. Maniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning, Cold Spring Harbor (1982).
9. H. Marquardt, A. Bendich, F. S. Phillips, and D. Hoffman, Chem. Biol. Interact., 3, 1 (1971).
10. S. J. McCready, D. A. Jackson, and P. R. Cook, Prog. Mutat. Res., 4, 113 (1982).
11. N. P. Napalkov, Transplacental Carcinogenesis, ed. by L. Tomatis and U. Mohr, Lyon (1973), pp. 1-13.
12. D. M. Pardoll, B. Vogelstein, and D. S. Coffey, Cell, 19, 527 (1980).

TIME COURSE OF FREQUENCIES OF SISTER CHROMATID EXCHANGE AND CHROMOSOMAL ABERRATIONS IN VIVO

S. V. Stukalov

UDC 612.6.052:575.224.23].014.46.66

KEY WORDS: thiotepa; rabbit; chromosomal aberrations; sister chromatid exchange.

During testing of the mutagenic activity of chemical substances in vitro, cell populations are studied during the period of mutagenic treatment and later under identical conditions. Under these circumstances, by the use of radioactive or BUdR labeling it is possible to monitor the rate of cell proliferation and the number of chromosomal aberrations (CA) and sister chromatid exchange (SCE) recorded in the cell [2, 5]. After exposure to mutagenic action in vivo the number and fraction of cells carrying SCE and CA diminishes with the course of time on account of selective death and proliferation of the cells, their redistribution in the tissues, and repair of injuries [6]. To determine the optimal times of taking blood samples after mutagenic action in vivo, and to choose the correct dose and interpret the results of cytogenetic analysis properly, it is essential to know the principles governing the time course of cells with cytogenetic lesions in vivo.

The aim of this investigation was to study changes in the frequency of SCE and CA with time after exposure to various doses of thiotepa (TP) in vivo.

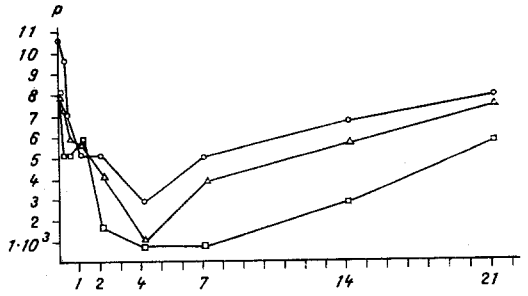

Fig. 1. Time course of number of nucleated cells (P, $10^3/mm^3$) in rabbits' blood. Here and in Figs. 2 and 3: circles — after injection of 2 mg/kg of TP; squares — 4 mg/kg, triangles — 6 mg/kg.

Institute of Medical Genetics, 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. 106, No. 8, pp. 220-221, August, 1988. Original article submitted May 14, 1987.

 0007-4888/88/0008-1168$12.50

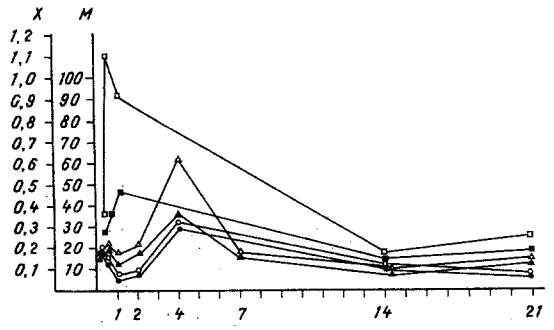

Fig. 2

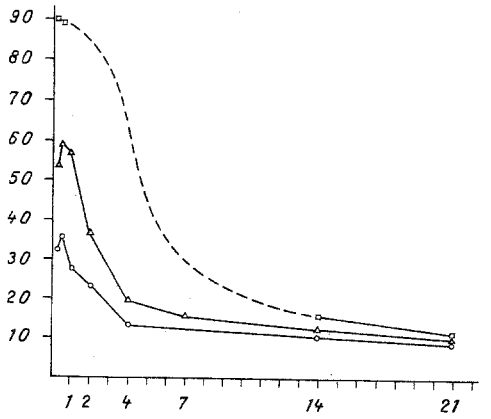

Fig. 3

Fig. 2. Time course of frequency of CA (M, filled symbols) and number of chromosomal breaks per cell(X, empty symbols).

Fig. 3. Time course of number or SCE.

EXPERIMENTAL METHOD

Three New Zealand White rabbits weighing 3-3.2 kg, aged 6 months, were used. An aqueous solution of TP was injected intravenously in doses of 2, 4, and 6 mg/kg. Before, and 6 and 9 h, and 1, 2, 4, 7, 14, and 21 days after injection of TP 2 ml of blood was withdrawn from a vein of the rabbit's ear, and the cells from it were cultured with BUdR for 65 h [1]. After preparation of metaphase chromosomes they were stained [4] and up to 100 cells were analyzed to determine the frequency of CA, and another 100 cells to determine the frequency of SCE. In each blood sample the number of nucleated cells in 1 mm^3 was determined.

EXPERIMENTAL RESULTS

It was shown previously that the number of CA and SCE in cells increases with time of observation during the first 6 h after a single injection of TP [3]. Knowing the rate of elimination of TP from the body, it was calculated that after injection of 2, 4, and 6 mg/kg of TP from substance was eliminated from the body in the course of 4.8, 8, and 11.3 h, respectively. Formulas for determining the maximal level of SCE and CA, based on the quantity of the substance injected, its elimination constant, and the sensitivity of the cells, were suggested previously [3]. After injection of 2, 4, and 6 mg/kg TP it was expected that the greatest number of chromosomal breaks per cell would be 0.134 (0.2023-0.0818), 0.329 (0.5227-0.1910), and 0.8120 (1.4216-0.4357) respectively (95% confidence intervals are given in parentheses). Experimentally, by the calculated time of total eimination of TP the highest values for the number of chromosomal breaks were 0.2, 0.22, and 1.11, respectively.

The calculated level of the peak SCE was expected to be 45.79 (±10.41), 88.05 (±17.24), and 144.34 (±26.34), respectively. The actual frequencies were 35.94 (±1.53), 59.13 (±2.30), and 89.93 (±2.89), respectively, i.e., with an increase in the quantity of substance injected the actual frequency of SCE differed increasingly from the expected value. After injection of large doses of TP (4 and 6 mg/kg) the frequency of SCE, after reaching a certain level, did not increase even if the mutagen was present in the body. This stabilization of the frequency of SCE may be associated with death of the cells, their selective proliferation in culture, and their redistribution in the body.

The number of nucleated cells in the animals' blood fell sharply in the first 4-7 days, then rose steadily (Fig. 1). On the 4th day, when the number of cells in the blood reached a minimum, an increase in the frequency of cells with CA and in the number of chromosomal breaks in the cells was observed (Fig. 2). However, at these times more than 36 metaphases could not be found for analysis, whereas after injection of 6 mg/kg of TP no dividing cells were obtained on the 2nd, 4th, and 7th days. By contrast with the curve showing the time course of CA, there was no increase in frequency on the 4th day in the time course of SCE (Fig. 3), and the frequency of SCE fell exponentially starting from 0.4 day, and was reduced by half on average after 9 days.

The increase in the frequency of CA on the 4th day was probably connected with release (rejection?) of the most severely damaged cells, incapable of division under ordinary

conditions, from the lymphopoietic organs. Under these circumstances, if cells not yet dividing in culture were used for analysis of CA, SCE were analyzed in cells which had gone through one division. Clearly cells with a large number of chromosomal lesions have a smaller chance of passing through mitosis, and for that reason, in the stage of culture selective proliferation of the less damaged cells takes place, and no increase in the number of SCE may necessarily be found.

During testing of a chemical substance for mutageneticity in vivo at least two factors must be taken into account: the time of taking the blood sample after injection of the substance and the quantity of substance injected (the dose of mutagenic action). If the time of taking the cells does not coincide with the time of complete elimination of the test substance from the body, underestimates of mutagenic activity can easily be obtained, for either the substances has not yet completed its action (early taking of the cells) or some cells with lesions have not yet reached the peripheral blood (late taking). In the present experiment the optimal dose of TP (from the point of view of determination of mutagenic activity) must be considered to be that with which there was a six-sevenfold rise in the level of SCE above the control. With larger doses the estimate of mutagenic activity in vivo may be distorted because of mass death of the cells in vivo or in culture.

LITERATURE CITED

1. N. P. Bochkov, S. V. Stukalov, and A. N. Chebotarev, Byull. Eksp. Biol. Med., No. 8, 90 (1982).
2. V. A. Sapacheva and K. N. Yakovenko, Genetika, 20, No. 1, 144 (1984).
3. S. V. Stukalov and A. N. Chebotarev, Byull. Éksp. Biol. Med., No. 11. 91 (1983).
4. A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova, Byull. Éksp. Biol. Med., No. 2, 242 (1978).
5. N. P. Bochkov, A. N. Chebotarev, T. V. Filippova, et al., Mutation Res., 127, No. 1, 149 (1984).
6. N. Huff, R. J. DuFrain, and L. G. Littlefield, Mutation Res., 94, No. 2, 349 (1982).