GENETICS

Sister Chromatid Exchanges at Different Times After Repeated Injections of Thiophosphamide *in Vivo*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 1, pp. 73-74, January, 1997
Original article submitted November 20, 1995

The frequency of sister chromatid exchanges in rabbit peripheral blood lymphocytes was estimated after three intravenous injections of thiophosphamide in doses of 0.5 and 2 mg/kg body weight at 2-week intervals. It is demonstrated that 24 h after the first injection the frequency is significantly higher than after subsequent injections.

Key Words: *sister chromatid exchanges; rabbit lymphocytes; thiophosphamide*

Previously, we showed the exponential decrease in the frequency of sister chromatid exchanges (SCE) after a single injection of mutagen has two phases: the fast (the first four days postinjection) and the slow (subsequent period) [1-3]. The first phase is associated with cell death, while the second phase may be related to the release of less damaged cells into the bloodstream and with reparation of the damage leading to SCE. In reality, living organisms are subjected to multiple exposure to clinical and environmental mutagens. Thus, general variations of cytogenetic effects resulting from repeated exposure to mutagens should be taken into account in the cytogenetic evaluation of the mutagenic load. Our purpose was to assess these variations.

MATERIALS AND METHODS

Two rabbits were used. They were injected intravenously three times with thiophosphamide (TP, aqueous solution) at 2-week intervals in a dose of 0.5 mg/kg (rabbit No. 1) and 2 mg/kg (rabbit No. 2). Blood was taken from the marginal ear vein before the first TP injection (baseline), 24 h after each injection, and 14 days before next injection. Lymphocytes were cultured with bromodeoxyuridine for 64-66 h, processed for the metaphase chromosomes, and stained for SCE. Forty cells per each time point were analyzed.

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RESULTS

Prior to the first TP injection, the number of SCE per cell was 6.1 in rabbit No. 1 and 6.08 in rabbit No. 2. The mean SCE frequencies on two different days after each TP injection are shown in Table 1. Comparison of these frequencies using the Student's t test showed that on day 14 postinjection SCE frequencies were significantly lower than on day 1. After administration of 0.5 mg/kg TP, there were no significant differences in the SCE frequencies on day 14 after the 1st, 2nd, and 3rd injection. However, at 2 mg/kg TP, the SCE frequency on day 14 after the 3rd injection was significantly higher than that after the 1st and 2nd injections (Table 1).

The ranges of SCE in different cells (figures in parentheses in Table 1) indicate that the decrease in

0007-4888/97/0001-0063$18.00

TABLE 1. Mean Frequencies of Sister Chromatid Exchanges (SCE) and Their Ranges (Figures in Parentheses) in Rabbit Lymphocytes at Different Times After Three Thiophosphamide (TP) Injections

TP dose, mg/kg	Day postinjection	TP injection		
		1st	2nd	3rd
0.5	1st	18.2 (12—33)	14.7 (7—26)	11.2 (6—17)
	14th	8.5 (3—14)	6.9 (2—15)	8.2 (3—16)
2	1st	49.7 (17—69)	42.2 (15—57)	32.1 (11—47)
	14th	9.1 (3—16)	10.2 (4—18)	14.7 (3—27)

the mean SCE frequency on day 14 after each TP injection was associated predominantly with the disappearance of cells carrying more exchanges and the emergence of cells in which the SCE frequency was similar or lower than the mean baseline value. In these cells, the damage leading to SCE had been repaired.

It was hypothesized [1] that cells containing 20 SCE do not die but remain in the bloodstream or hemopoietic organs to repair the damage. The reparation requires several cell cycles, as evidenced by the emergence of cell clones with intermediate frequencies of SCE which were not observed immediately before or after TP administration. For example, the SCE frequency was higher on day 14 after 2 mg/kg TP than after 0.5 mg/kg TP, because the cell cycle of cells with more SCE is longer and reparation is less efficient.

Previously, we showed that the expected frequencies of cytogenetic effects can be calculated from the dose of the mutagen, the rate of its elimination, and cell sensitivity to it [3]. In the present study, the SCE frequencies 24 h after the 1st TP injection (0.5 or 2 mg/kg) were very close to the calculated frequencies, whereas those observed 24 h after the 2nd and 3rd injections were significantly lower. Since each time the rabbits received the same dose of TP, the rate of TP elimination (metabolic inactivation) and/or the sensitivity of cells to the mutagen changed after each injection.

The decrease in the SCE frequency recorded 24 h after each TP injection may be associated with alterations of the reparation processes. However, it is unclear how the reparation of the damage leading to SCE is influenced by mutagens [4]. For the correct data interpretation, the complex relationship between reparation and SCE formation as well as the mutagen dose, type of mutagen and intervals between exposures should be taken into consideration. One should remember that in addition to cell death and reparation of cell damage, the effect of the mutagen changes due to the compensatory increase in proliferative activity of hemopoietic cells. This increase was observed on days 3-4 after a massive death of peripheral blood lymphocytes [2].

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