COMPARATIVE FREQUENCIES OF SISTER CHROMATID EXCHANGES INDUCED BY THIOPHOSPHAMIDE *IN VIVO* AND *IN VITRO*

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KEY WORDS: sister chromatid exchanges; thiophosphamide.

Some mutagenic substances are known to increase the number of sister chromatid exchanges (SCE) whether the target cells are treated *in vivo* or *in vitro*. About 67% of substances tested for mutagenicity induce both chromosomal aberrations (CA) and SCE [4]. As yet the question of dependence of changes in SCE and CA *in vivo* and *in vitro* and the possibility of extrapolating results of *in vitro* studies to *in vivo* conditions have not been investigated. Previously [1] the writers published the results of an analysis of induction of CA by thiophosphamide in rabbit lymphocytes treated *in vivo* and *in vitro* and showed that the dose dependence of the change in frequency of CA was the same in both systems.

The aim of the present investigation was to study induction of SCE by thiophosphamide *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

Thiophosphamide, a highly active alkylating compound with a mutagenic action even without preliminary metabolic activation, was chosen as the mutagen. Experiments *in vivo* and *in vitro* were carried out on the same gray Chinchilla rabbits. Three rabbits were used. Since the procedures involved only minimal pain, general anesthesia was not used. The experiments *in vitro* preceded those *in vivo*.

Methods of treating the cells with thiophosphamide *in vivo* and *in vitro*, of culture after treatment, and fixation and preparation of the films were similar to those described previously [1]. To induce SCE *in vitro* the cells were incubated for 1 h at 39°C with thiophosphamide in concentrations of 2, 4, 6, 8, and 10 μg/ml, and in the control tests Hanks' solution was added. In the experiments *in vivo* an aqueous solution of thiophosphamide was injected intravenously in a dose of 3, 5, 7 mg/kg body weight. During the 360 min after the injection blood smaples were taken from the animals to determine the concentration of thiophosphamide [2] and in order to culture lymphocytes after preliminary washing of the cells to remove traces of mutagen. The cells were fixed at the 68th-70th hour of culture in experiments both *in vivo* and *in vitro*.

The frequencies of SCE were analyzed in numbered slides, under a magnification of 900 times of the microscope, in metaphase plates of the 2nd mitotic division, with harlequin staining of the chromosomes. Metaphase plates with 43-44 chromosomes were analyzed. After each kind of treatment 25 plates were analyzed. The number of changes in staining along the length of the chromatid was taken as the number of SCE.

The method of determining the dose of mutagenic action was similar to that described previously [1]. In experiments *in vivo* dose was determined as the integral of the function of change in thiophosphamide concentration in the animal's blood from the time of injection of the agent until the time of taking the blood sample. In experiments *in vitro*, the dose was taken as the product of thiophosphamide concentration and duration of its action (60 min), for the concentration of the mutagen remained unchanged during the incubation time.

EXPERIMENTAL RESULTS

The main aim during induction of SCE *in vivo* and *in vitro* was to compare dependence of the change in frequency of SCE on an increase in dose of mutagenic action in both cases. Al-

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TABLE 1. Results of Regression Analysis of Changes in Frequency of SCE with a Change in Dose ($S = b_0 + b_1 \cdot D$)

No. of rabbits	In vitro				In vivo			
	r^2	b_0	b_1	F_p	r^2	b_0	b_1	F_p
1	0,968	7,1200 (±9,2279)	0,09340 (±0,03139)	89 $P<0,001$	0,907	11,8044 (±8,1243)	0,06120 (±0,02723)	39 $P>0,01$
2	0,985	7,5219 (±5,2199)	0,08277 (±0,01437)	256 $P<0,001$	0,966	16,0992 (±6,6578)	0,09809 (±0,02122)	141 $P>0,001$
3	0,976	8,5524 (±7,1688)	0,09125 (±0,01973)	165 $P<0,001$	0,983	17,1111 (±8,6402)	0,07371 (±0,01359)	227 $P>0,001$
Total for three rabbits	0,967	7,8810 (±3,0685)	0,08820 (±0,00891)	444 $P>0,001$	0,915	15,7330 (±5,5485)	0,07626 (±0,01233)	172 $P>0,001$

Legend. 95% confidence limits shown in parentheses.

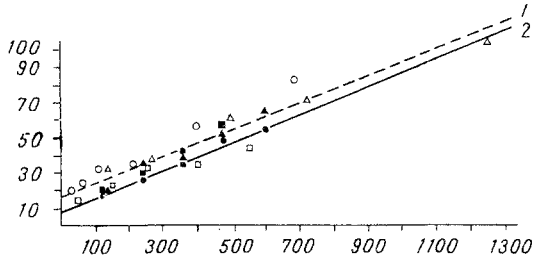

Fig. 1. Regression lines and experimental points of change in frequency of SCE with increase in mutagenic action *in vivo* (1) and *in vitro* (2). Empty symbols denote experimental values of SCE determined *in vivo*, filled symbols — the same, *in vitro*. Abscissa, dose of thiophosphamide (in μg/ml·min); ordinate, number of SCE per cell.

together 1000 cells were analyzed. The experimental data on induction of SCE, in the form of mean values of SCE for each type of treatment and each dose of mutagenic action, were subjected to regression analysis. It was assumed that the increase in the number of SCE is a linear function of the increase in dose [3], i.e., that it has the form $S = b_0 + b_1 \cdot D$, where S is the frequency of SCE for a dose D, b_0 is the spontaneous level of SCE obtained by extrapolating the regression line to zero dose, and b_1 is the angular coefficient of the regression line, values of which were used to compare the regression line for conditions *in vivo* and *in vitro*.

The results of regression analysis are given in Table 1.

As Table 1 shows, the explainable scatter fraction (r^2) was high in all cases (not below 0.907) and values of b_0 and b_1 lie within the same 95% confidence interval both for each rabbit in the two systems and between individuals, so that it was possible to undertake regression analysis for all three rabbits together, separately for *in vivo* and *in vitro* systems. For the experiments *in vivo* the increase in the number of SCE with an increase in dose is given by: $S = 15.7330(\pm 5.5485) + 0.07626(\pm 0.01233) \cdot D$, and for the experiments *in vitro*: $S = 7.8810 (\pm 3.0685) + 0.08820 \times (\pm 0.00891) \cdot D$ (95% confidence limits are given in parentheses). Regression lines satisfying the above equations are almost parallel (Fig. 1).

It can be concluded from these results that thiophosphamide induces SCE equally effectively *in vivo* and *in vitro*. These results, and also data published previously [1] on analysis of CA induced by thiophosphamide in rabbit lymphocytes are evidence that the results of analysis of SCE and CA after induction *in vitro* can be extrapolated to a system *in vivo*. An adequate and uniform approach to determination of the dose of mutagenic action must be observed under these circumstances, the target cells must be derived from the same tissue, and frequencies of CA and SCE must be analyzed soon after injection of the agent *in vivo*.

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TRANSMISSIVITY RANGE OF GENETIC TRANSFER FACTORS pAP38, pAP39, pAP41, and pAP42

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The F-like genetic transfer factors pAP38, pAP39, pAP41, and pAP42 were identified in cells of strains of *Escherichia coli* isolated from man and animals [3], and their molecular weights are 49.3×10^6, 42.6×10^6, 40.0×10^6, and 32.2×10^6 daltons, respectively. Whereas transfer factors pAP38 and pAP42 are representatives of FVII and FIX incompatibility groups, respectively, factors pAP39 and pAP41 have atypical incompatibility and belong simultaneously to incompatibility groups FI and FIV [1, 2].

The writers showed previously that these transfer factors can be transmitted into cells of untyped and typed strains of *E. coli* and also into bacteria of the genera *Erwinia* and *Hafnia*, in which they are also able to replicate.

The aim of the present investigation was to study the ability of all these genetic transfer factors to be transmitted into bacteria of more distant species and to express themselves in these bacteria.

EXPERIMENTAL METHOD

Genetic transfer factors pAP38, pAP39, pAP41, and pAP42, marked by transposon Tn9 (pAP38::Tn9, pAP39::Tn9, pAP41::Tn9, pAP42::Tn9) and contained in cells of *E. coli* AP115, mutant factor pAP38-2drd, marked with this same transposon (pAP38-2drd::Tn9) and contained in *E. coli* 132, and also conjugative plasmid RP4ApNmKmTc, contained in *E. coli* AE, were used in the experiments.

The transmissivity range of the transfer factors was studied by using cells of strains *Pseudomonas putida* BKMB-901, *Pseudomonas fluorescens*, *Rhizobium leguminosarum* BKMB-115, *Azospirillum lipoferum*, and *Agrobacterium tumefaciens* as recipients.

Donor cells of *E. coli* AP115 and AP132, containing one or the other transfer factor, were crossed with recipient cells of *Ps. putida*, *Ps. fluorescens*, and *A. tumefaciens* by standard methods in nutrient both followed by selection of transconjugants on nutrient agar containing chloramphenicol and streptomycin. Crosses in which *A. lipoferum* or *R. leguminosarum* cells served as receipients were carried out on solid potato medium or agarized synthetic medium, also with the addition of chloramphenicol and streptomycin. "Three-parent" crosses were carried out by the method described previously [3]. The sensitivity of bacteria containing plasmids pAP42 and RP4 to phages MS2 and PRD1 was determined by the agar layers method.

EXPERIMENTAL RESULTS

Despite many repetitions of the experiments, no transconjugants could be isolated from a single cross in which *E. coli* AP115 or AP132 cells containing one or the other genetic trans-

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