almost up to the control values; differences between MM in the experiment and control were not statistically significant ($P > 0.05$). Practically normal sedimentation profiles of DNA were obtained also for cells incubated after exposure to bleomycin for 3 and 24 h. This means that cells of 101/H mice in culture can repair single-stranded DNA breaks induced by bleomycin very effectively.

A defect of DNA repair, manifested in 101/H mice as impaired ability to remove lesions of the DNA–DNA or DNA–protein cross-linkage type [11], thus does not affect the efficiency of repair of single-stranded DNA breaks. It can thus be taken as established that with respect to this feature, cells of 101/H mice do not differ from those of patients with chromosomal instability and with a defect of excision repair of DNA.

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COMPARISON OF THE FREQUENCY OF CHROMOSOMAL ABERRATIONS INDUCED BY THIOPHOSPHAMIDE IN RABBIT LYMPHOCYTES *IN VITRO* AND *IN VIVO*

N. P. Bochkov,* S. V. Stukalov, and A. N. Chebotarev

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The question of comparability of data on mutagenesis obtained *in vivo* and *in vitro* and the possibility of extrapolating data from one system to the other has frequently been discussed in the literature. Most investigations devoted to comparison of the mutagenic action of chemicals *in vivo* and *in vitro* have been qualitative in character, i.e., devoted to the study of mutagenic activity of a certain substance *in vivo* and *in vitro*, but not concerned with the degree of manifestation of this activity in the two systems. This problem can be studied more fruitfully in the field of radiation mutagenesis by the use of the chromosomal aberrations (CA) test [1]. In chemical mutagenesis, the problem of comparison of the cytogenic

*Academy of Medical Sciences of the USSR.

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

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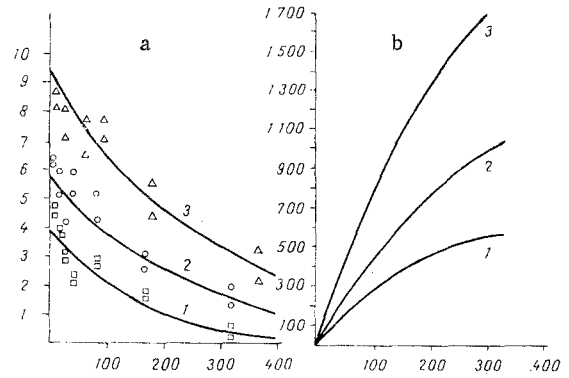

Fig. 1

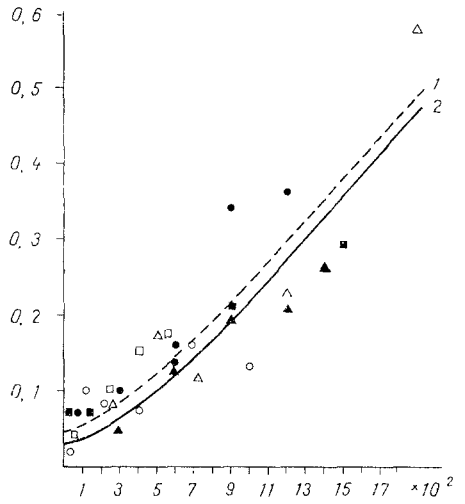

Fig. 2

Fig. 1. Changes in blood thiophosphamide concentration as a function of dose used for mutagenic action *in vivo*: a) change in blood thiophosphamide concentration; 1) excretion curve and experimental points for rabbit No. 1 after injection of 3 mg/kg thiophosphamide; 2) the same for rabbit No. 2, 5 mg/kg injected; 3) the same for rabbit No. 3, 7 mg/kg injected; b) increase in mutagenic exposure dose for lymphocytes *in vivo*: 1) curve for rabbit No. 1; 2) for rabbit No. 2; 3) for rabbit No. 3. Abscissa, time (in min); ordinate: a) thiophosphamide concentration (in μg/ml); b) mutagenic exposure dose (in μg/ml/min).

Fig. 2. Dependence of fraction of aberrant mitoses on mutagenic exposure dose *in vivo* (1) and *in vitro* (2). Empty symbols indicate experimental points of experiments *in vivo*, filled symbols — the same *in vitro*. Abscissa, mutagenic exposure dose (in μg/ml/min); ordinate, fraction of aberrant mitoses.

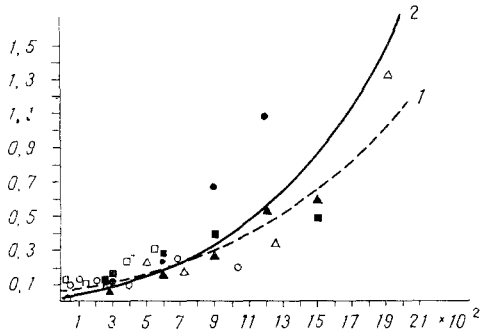

Fig. 3. Dependence of number of breaks per cell on mutagenic exposure dose. Ordinate, number of breaks per cell. Remainder of legend as in Fig. 2.

effects induced *in vivo* and *in vitro* can be solved more or less successfully only by analysis of sister chromatid exchanges (SCE). There have been very few studies to compare the frequency of CA induced *in vivo* and *in vitro*, and like those devoted to analysis of SCE, all these investigations have the same shortcomings, which prevent any strict quantitative comparison of results obtained *in vivo* and *in vitro*.

The aim of the present investigation was to compare the frequency of CA induced by thiophosphamide in rabbit blood lymphocytes *in vivo* and *in vitro* and to test experimentally the suggested approach to the determination of the mutagenic exposure dose.

EXPERIMENTAL METHOD

Thiophosphamide, a highly active trifunctional alkylating compound, not requiring metabolic activation to exhibit its mutagenic effect, was used as the mutagen. Experiments *in vivo* and *in vitro* were conducted on the same rabbits of the Chinchilla breed, experiments *in vitro* preceding those *in vivo*.

In the experiments *in vitro*, 6-7 ml blood was collected in a sterile heparinized syringe from the auricular vein and transferred in doses of 0.5 ml into tubes containing 8.5 ml of medium 199. After incubation of the cell suspension for 30 min at 39°C, 1 ml of solutions of mutagen of different concentrations was added to the tubes up to a final concentration of 5, 10, 15, 20, and 25 μg/ml in the incubation mixture for 1 h. Hanks' solution in a volume of 1 ml was added to the control tubes. After treatment, the suspension was washed twice with cold Hanks' solution.

In the experiments *in vivo*, 5-7 ml of thiophosphamide solution was injected intravenously into the animals in a dose of 3 mg/kg for rabbit I, 5 mg/kg for rabbit II, and 7 mg/kg for rabbit III. Blood samples (about 2 ml) were taken with a heparinized syringe from the auricular vein 5, 10, 20, 40, 80, 160, and 320 min after injection of the thiophosphamide, and each sample was divided into two parts. One part was used to determine the thiophosphamide concentration in the blood at that given moment of time (two measurements) by the method published previously [2]. The other part of the blood was used to obtain a lymphocyte culture. For this purpose, the blood samples were first washed 3 times with 30 volumes of cold Hanks' solution. Blood samples were taken before injection of thiophosphamide to analyze the level of spontaneous CA in the animals.

Thereafter, the methods of culture, obtaining the preparations and staining them, were the same in the experiments *in vivo* and *in vitro*. To 0.5-0.7 ml of the washed suspension of blood cells, a culture mixture consisting of 3 parts medium 199 and 1 part homologous (rabbit) serum was added up to a volume of 8 ml. Concanavalin A was added to the culture mixture to a final concentration of 15 μg/ml. To identify mitoses I, II, and III, 5-bromodeoxyuridine was added to the culture medium to a final concentration of 10 μg/ml after the beginning of culture. The suspension was transferred into 50-ml vitamin flasks, which were tightly closed with rubber stoppers and wrapped in brown paper to keep out the light. The cultures were fixed after 56 h. To collect mitoses, colchicine was added 2 h before fixation up to a final concentration of 0.5 μg/ml. The cells were subjected to hypotonic treatment with KCl solution containing 7 g/liter at 37°C for 7 min and fixed with a cold mixture of methanol and acetic acid (3:1). After three changes of fixative, the cell suspension was dropped on to cold wet slides and dried without flaming the fixative.

To detect differential staining of sister chromatids, the preparations were stained by the method in [3]. The first mitoses with evenly stained chromosomes were analyzed on numbered slides.

EXPERIMENTAL RESULTS

The dose was determined by the equation

$$D = \int_0^t f(C)\,dt.$$

Since the thiophosphamide concentration during the first hour in the experiments *in vitro* remained constant, it follows that $f(C) = \text{const} = C_0$ and, consequently, $D = C_0 \cdot t$ μg·min/ml, where C_0 is a concentration of between 5 and 25 μg/ml and t the time which in all experiments *in vitro* was 60 min. In the experiments *in vivo*, the blood thiophosphamide concentration fell exponentially with time, and for that reason its concentration had to be measured in the course of the experiment. For all rabbits, changes in concentration were satisfactorily described by the equation

$$C = C_0 e^{-st}.$$

The results of measurement of the thiophosphamide concentrations and curves calculated by the method of least squares in accordance with the equation given above are shown in Fig. 1a (for rabbit I r^2 was 0.93, for rabbit II 0.91, and for rabbit III 0.91). Using the values of s and C_0 thus found for all the rabbits, exposure doses of thiophosphamide for lymphocytes *in vivo* were calculated by the equation

$$D = \int_0^t C_0 e^{-st} dt = \frac{C_0}{s}(1 - e^{-st}).$$

Curves showing changes in the value of the dose with the duration of exposure *in vivo* are given in Fig. 1b.

The frequency of CA was determined during the action of several doses both *in vitro* and *in vivo*, in order to judge the character of the response on the basis of a set of points described by the dose–effect curve. Changes in the frequency of aberrant metaphases ρ with an increase in dose in the experiments *in vitro* are satisfactorily described by the equation $\rho = 1-e-(^{-kD+}\alpha)^2$ ($r^2 = 0.7886$, $F_{inad} = 1.80$), and the number of chromosomal breaks per cell (X) is described by the equation $X = e^{(kD+\alpha)^2}-1$ ($r^2 = 0.8039$; $F_{inad} = 2.23$). For aberrant metaphases: $k = 0.0003309$ (± 0.0000982), $\alpha = 0.1644$ (± 0.0820) — 95% confidence limits are given in parentheses. The predicted spontaneous frequency of aberrant metaphases $\rho = 2.67\%$ ($\pm 0.67\%$). For the number of chromosomal breaks per cell, $k = 0.0004091$ (± 0.0001158), $\alpha = 0.1742$ (± 0.0967), the expected number of chromosomal breaks per cell in the control $X_0 = 0.0308$ (± 0.0094). Changes in the number of aberrant metaphases and in the number of breaks per cell in the experiments *in vivo* can be described by the same equations as in the experiments *in vitro*. For aberrant metaphases: $r^2 = 0.829$, $k = 0.000328$ (± 0.000070), $\alpha = 0.1995$ (± 0.0429); $\rho_0 = 3.90\%$ ($\pm 0.18\%$). For the number of chromosomal breaks per cell: $r^2 = 0.807$, $k = 0.0003220$ (± 0.0000736), $\alpha = 0.2280$ (± 0.0454), $X_0 = 0.0533$ (± 0.0021).

Since the value of k reflects the degree of correlation between effect and dose, the results obtained are evidence that, first, dose dependences for thiophosphamide *in vivo* and *in vitro* are of the same character and, second, within 95% confidence limits the same exposure dose *in vivo* and *in vitro* gives rise to the same effect, as is shown by Figs. 2 and 3.

In the course of analysis of the experimental data, the spectrum of aberrations *in vivo* and *in vitro* was studied. The ratio between the number of chromatid breaks and all chromosomal breaks was found to stay constant, and for experiments *in vivo* within the dose range from 100 to 1900 μg·min/ml it was 0.82 and for experiments *in vitro* within the dose range from 300 to 1500 μg·min/ml the fraction was 0.83. Changes in the ratio between the number of breaks involved in exchange aberrations to the total number of chromosomal breaks also were analyzed with an increase in dose between the same limits. The fraction of exchange aberrations was found to be roughly the same in experiments *in vivo* and *in vitro* (0.52 and 0.60, respectively). No differences between exposure *in vivo* and *in vitro* could likewise thus be found with respect to the spectrum of chromosomal aberrations.

The results suggest a direct approach for comparison of mutagenic action *in vivo* and *in vitro*. On the basis of the pharmacokinetics of a substance *in vivo* or a change in its concentration *in vitro*, the dose numerically equal to the area lying beneath the curve of change in concentration is determined. Since the effectiveness of action of the same dose is the same *in vivo* and *in vitro*, direct extrapolation of the *in vitro* results to the situation *in vivo* is possible provided that the same tissue is used in both experiments.

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