DEPENDENCE OF FREQUENCIES OF CYCLOPHOSPHAMIDE-INDUCED CHROMOSOMAL ABERRATIONS AND SISTER CHROMATID EXCHANGES ON MUTAGEN DOSE *IN VIVO* AND *IN VITRO*

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One possible way of predicting genetic consequences of human exposure to external environmental factors is based on the analogy with experimental objects, but in this case difficulties associated with the extrapolation of data obtained *in vitro* to conditions pertaining *in vivo* must be taken into account [1]. It was shown previously that if rabbit lymphocytes are treated with thiophosphamide *in vivo* and *in vitro* the same dose-dependence of the increase in frequency of chromosomal aberrations (CA) [2] and of sister chromatid exchanges (SCE) [4] is observed *in vivo* and *in vitro*; thiophosphamide had the same cytostatic action under these circumstances on lymphocytes *in vivo* and *in vitro* [5]. Direct quantitative comparison of the results of mutagenesis *in vivo* and *in vitro* in this way became possible by defining the dose of mutagenic exposure (D) in such a way as to take into account both the change in the concentration of the substance in the environment (S) and the duration of action of the mutagen on the cell: $D = \int_0^T C(t)\,dt$. However, thiophosphamide is a mutagen with direct action, and the main difficulty of the study of dose dependence in chemical mutagenesis is the fact that metabolic conversions of chemical substances in living systems must be taken into account.

Accordingly, the aim of the present investigation was to establish to what extent the approach to determination of the dose of mutagenic exposure indicated above is applicable for an indirect mutagen, namely cyclophosphamide (CP), which undergoes a number of metabolic changes *in vivo*, with the formation of mutagenic products [8, 9].

EXPERIMENTAL METHOD

Experiments were carried out *in vivo* and *in vitro* on New Zealand rabbits. Before the animals were given CP, 8-10 ml of blood was taken from their auricular vein for the control tests and for the experiments *in vitro*. CP (Ministry of the Medical Industry, Saransk), in a dose of 200 mg, was dissolved in 5 ml of distilled water and injected intravenously in doses of 30 mg/kg (rabbit No. 1) and 20 mg/kg (rabbits Nos. 2-4). In the course of 245-350 min blood was taken 5-7 times from the vein in a volume of 3 ml each time, and some of it was used to determine the mutagenic effect *in vivo*, the rest to determine the alkylating activity of CP metabolites in the plasma (the NBP test) [3].

In the experiments *in vitro* lymphocytes were incubated with plasma, obtained from the blood of the same animals but taken 50-70 min after injection of CP, and containing active metabolites of CP. "Active" plasma in a volume of 1 to 4 ml in different experimental variants was added to 1 ml of intact blood and the volume was made up to 5 ml with Hanks' solution. The samples were incubated at 39°C for 4-6 h.

Lymphocytes washed free from the mutagen were cultured for 66-68 h in a culture mixture consisting of 0.5 ml of blood, 6 ml of Eagle's medium with glutamic acid, and 1.5 ml of rabbit

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TABLE 1. Frequency of Chromosomal Aberrations and SCE During Exposure to CP *in Vivo* and *in vitro*

Rabbit No.	*in Vivo*				*in Vitro*			
	Dose, c.u.	Number of SCE per cell	ρ	χ	Dose, c.u.	Number of SCE per cell	ρ	χ
1	0	6,44±0,1	—	—	0	6,20±0,11	—	—
	7,38	15,92±0,14	—	—	4,13	12,64±0,15	—	—
	17,59	25,68±0,34	—	—	8,26	17,76—0,17	—	—
	19,39	37,60±0,37	—	—	13,22	25,92±0,22	—	—
2	0	5,16±0,09	0	0	0	5,16±0,09	0	0
	5,50	17,22±0,26	0,05	0,07	6,30	16,88±0,18	0,03	0,04
	9,14	22,72±0,22	0,11	0,13	12,70	20,72±0,30	0,07	0,10
	11,58	30,08±0,36	0,15	0,22	16,76	27,00±0,23	0,09	0,17
	13,03	—	0,15	0,23				
3	0	7,16±0,12	0,01	0,02	0	7,16±0,12	0,01	0,02
	6,16	19,64±0,23	—	—	6,31	19,64±0,21	0,13	0,15
	10,59	26,00±0,32	0,09	0,11	12,62	30,24±0,22	0,13	0,17
	13,34	28,52±0,19	0,11	0,15	20,20	37,68±0,26	0,16	0,24
	14,79	30,25±0,29	0,14	0,19	—	—	—	—
4	0	7,56±0,10	0,01	0,01	0	7,56±0,10	0,01	0,01
	3,80	16,28±0,15	0,08	0,08	3,10	13,36±0,41	0,03	0,04
	7,35	20,36—0,16	0,05	0,06	6,20	20,44±0,17	0,07	0,07
	10,13	23,80±0,28	0,09	0,12	9,91	29,56±0,25	0,08	0,11
	11,39	25,80±0,30	0,13	0,19	—	—	—	—

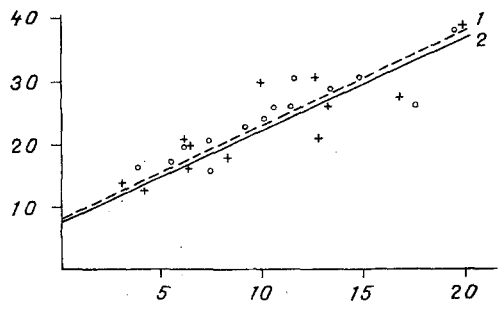

Fig. 1

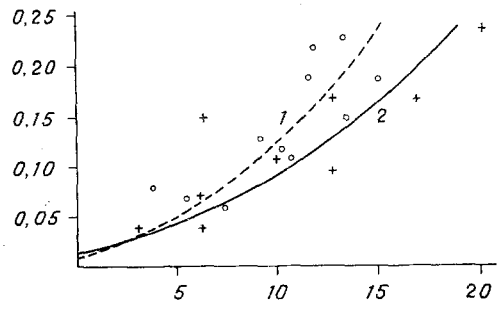

Fig. 2

Fig. 1. Dependence of number of SCE on dose of mutagenic exposure *in vivo* (1) and *in vitro* (2). Abscissa, dose (in c.u.); ordinate, number of SCE per cell. Circles denote experimental values of mean number of SCE in experiments *in vivo*, crosses denote the same, *in vitro*.

Fig. 2. Dependence of number of chromosomal breaks on dose of mutagenic exposure *in vivo* and *in vitro*. Ordinate, number of chromosomal breaks per cell. Remainder of legend as to Fig. 1.

serum with the addition of concanavalin A and bromodeoxyuridine to a final concentration of 15 and 10 μg/ml, respectively. The cells were fixed and specimens prepared and stained by methods described previously [2, 6].

CA were analyzed in 1st division metaphases, SCD in 2nd division metaphases (100 and 25 cells, respectively, were analyzed for each dose).

Just as in the experiments with thiophosphamide, the dose of mutagenic exposure to CP *in vivo* was determined as the area beneath the curve showing the change in the blood levels of the substance. An equation for the kinetics of active CP metabolites *in vivo*, suggested by Brock [8], was used for this purpose:

$$y = \frac{k_1}{k_1 - k_2} y_0 \left(e^{-k_2 t} - e^{-k_1 t}\right).$$

The parameters k_1, k_2, and y_0 were determined for experimental points (obtained by the NBP test) by the method of least squares (MLS). Unlike the experiments with thiophosphamide, the change in the concentration of CP metabolites in the blood was complex in character. During the first 60 min the concentration of metabolites rose quickly, then fell during the next 4-6 h.

When dose was determined *in vitro*, the fall in concentration of the mutagen during incubation was taken into account. The dose was expressed in conventional units (c.u.) as the product of optical density and duration of action (dimensionality: optical density units × min).

EXPERIMENTAL RESULTS

In the study of dose-dependence of the change in the number of SCE about 900 cells were analyzed (Table 1). Dependence of the mean number of SCE per cell on dose was assessed by regression analysis. Better agreement was obtained with a linear model ($F \geqslant 103$, $R^2 \geqslant 0.9$). It can be concluded from the considerable overlapping of the 95% confidence intervals for angular coefficients of regression (b_1) that the effectiveness of mutagenic CP metabolites in inducing SCE was the same *in vivo* and *in vitro* ($b_1 = 1.47 \pm 0.25$ and $b_1 = 1.45 \pm 0.31$, respectively). Experimental points and regression lines obtained by analysis of the pooled data for four animals are shown in Fig. 1. During analysis of dose-dependence of the change in the number of CA about 2300 cells were analyzed (Table 1). To approximate dependence of the number of chromosomal breaks (X) and the fraction of aberrant metaphases (ρ) on the dose of CP the following equations [7] were used:

$$X = e^{(\alpha+kD)^2} - 1; \quad \rho = 1 - e^{-(\alpha+kD)^2}.$$

Unknown coefficients, both *in vivo* and *in vitro*, were chosen by the MLS method. It will be clear from analysis of the data that although the values of the coefficients for the experiments *in vivo* and *in vitro* overlapped in their 95% confidence interval ($k = 0.024 \pm 0.006$ and $k = 0.019 \pm 0.007$ for X, $k = 0.022 \pm 0.006$ and $k = 0.016 \pm 0.008$ for ρ, respectively), nevertheless, the rate of rise of the frequency of CA was somewhat higher *in vivo* than *in vitro*. This is particularly noticeable over the range of high doses (Fig. 2).

CP had not only a mutagenic action on rabbit lymphocytes, but also a marked cytostatic action, as was shown by a decrease in the frequency of the 2nd, 3rd, and subsequent mitoses with an increase in dose *in vivo* and *in vitro*.

Hence, just as in the case of the direct mutagen thiophosphamide, so also for indirect mutagens of the CP type, direct quantitative comparison of the results of mutagenesis *in vivo* and *in vitro* is possible on the basis of the uniform and adequate determination of the dose of mutagenic exposure in both systems.

LITERATURE CITED

1. N. P. Bochkov, Vestn. Akad. Med. Nauk SSSR, No. 3, 13 (1981).
2. N. P. Bochkov, S. V. Stukalov, and A. N. Chebotarev, Byull. Eksp. Biol. Med., No. 8, 90 (1982).
3. O. P. Kirichenko, A. N. Chebotarev, and K. N. Yakovenko, Byull. Eksp. Biol. Med., No. 5, 552 (1976).
4. S. V. Stukalov and A. N. Chebotarev, Byull. Eksp. Biol. Med., No. 4, 74 (1983).
5. S. V. Stukalov and A. N. Chebotarev, Byull. Eksp. Biol. Med., No. 12, 89 (1983).
6. A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova, Byull. Eksp. Biol. Med., No. 2, 242 (1978).
7. A. N. Chebotarev and K. N. Yakovenko, Genetika, No. 8, 150 (1975).
8. N. Brock, R. Gross, H.-J. Hohorst, et al., Cancer (Philadelphia), 27, 1512 (1971).
9. B. E. Domeyer and N. E. Sladek, Cancer Res., 40, 174 (1980).